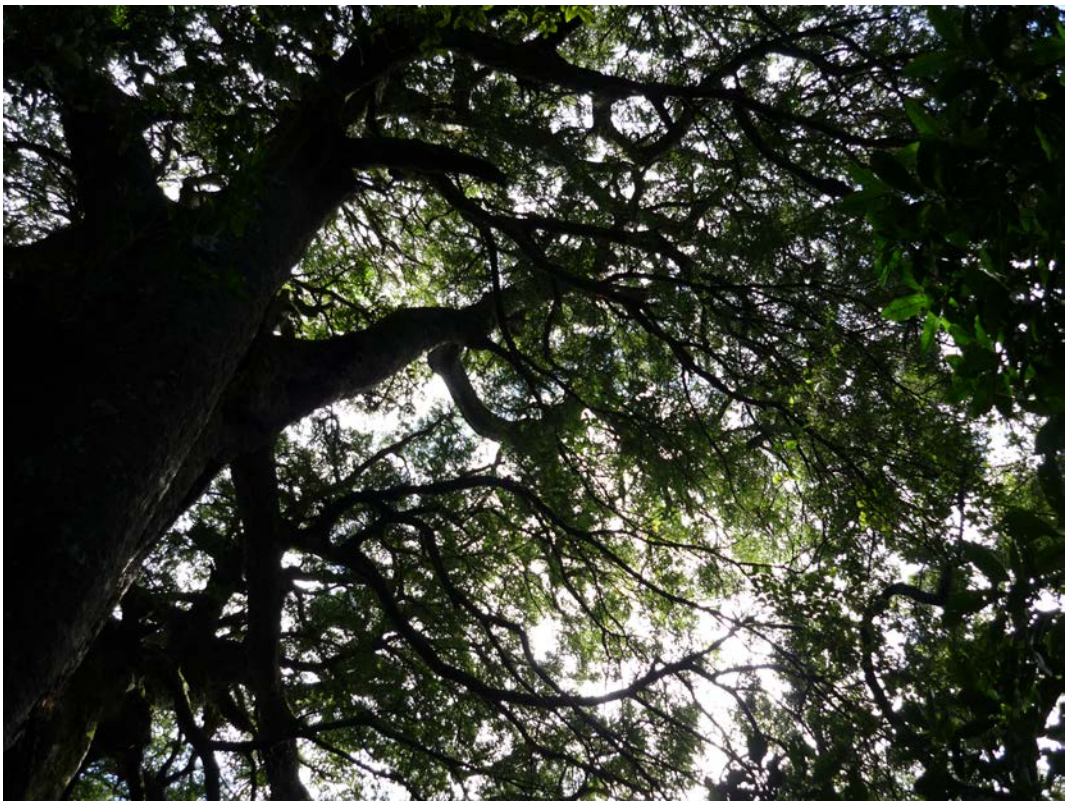
Quantifying seed dispersal of matai (*Prumnopitys taxifolia*)



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Michael John O'Connor

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He lit the fire, but taught me to gather my own fuel.

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Abstract

In New Zealand's highly modified environment, it is unclear how ecological services function within modified habitats and with an altered suite of mutualists and predators. I used matai (*Prumnopitys taxifolia*) to investigate the status of dispersal including the range of native and introduced species involved, and to test the feasibility of using microsatellites (repetitive regions of DNA) to identify maternal origin of seeds and therefore measure dispersal. I isolated ten microsatellite loci, five of which were polymorphic. Across 20 adult matai on Banks Peninsula, none shared a multi-locus genotype across these five loci; this suggests that these loci will be sufficiently variable to allow the individual identification required for parentage analysis. Although logistic problems prevented full testing of the system, preliminary results suggest that these microsatellites will allow identification of the maternal parent of seeds genotyped from their seed coat. Seed traps and ground plots throughout forest fragments on the Port Hills found varying levels of insect (0–19%) and rodent (0–1.5%) pre-dispersal predation over two years; despite this, 19–55% of seeds caught beneath female matai trees had been consumed and excreted by a bird. The majority of seeds (98%) were caught within 55 m of the nearest female matai tree, with a maximum distance of 130 m, indicating dispersal was occurring within these sites. Through observations on fruiting trees, I found four bird species regularly visited and fed on matai fruits; native kereru (*Hemiphaga novaeseelandiae*) and bellbird (*Anthornis melanura*), and exotic blackbird (*Turdus merula*) and song thrush (*T. philomelos*). As expected, kereru performed the majority of feeding visits (50%), but surprisingly the two introduced species combined contributed 22% of the feeding visits. I argue that the prevailing methods for measuring avian frugivory systematically under-estimate the contribution made by introduced birds. This finding is important as *Turdus* species are abundant and widespread in New Zealand. Finally, I examined whether feral pigs (*Sus scrofa*) disperse seeds of matai. Both captive and wild pigs consumed matai seeds and excreted a proportion of these intact; germination rates of these seeds (57–68%) were comparable to hand-cleaned seeds (64%). This is the first demonstration of feral pigs dispersing the seeds of a New Zealand native plant. Large mammals such as pigs may provide long distance dispersal of fleshy-fruited species, which will be particularly important in today's highly fragmented forests. Further dispersal research should consider the effects of these introduced avian and mammalian dispersers if we are to understand how ecological services are functioning amidst our modified environments.

Preface

Except where noted below, all the experimental work, data analysis and writing in this thesis is my own. I took all photographs included throughout the thesis. All maps were drawn in ArcMap v9.1 (ESRI).

Chapter 2 covers the development of microsatellite primers. 454 sequencing was conducted under contract by Otago Sequencing. Genotyping was conducted by Canterbury Sequencing. I conducted all other laboratory work.

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Chapter 5 has been published in the *New Zealand Journal of Ecology*, but has been presented here in the same format as the rest of the thesis for consistency; the published version is provided in Appendix B. This paper is co-authored with my senior supervisor, Prof. Dave Kelly. Prof. Kelly suggested the research based on observations he had made in the study site (Isolated Hill) some years prior. I conducted all of the field work, lab processing of seeds and pig faeces, and germination experiments. I set up the germination experiments and monitored them except for help from Jenny Ladley during two spells I was overseas in 2008 and 2009. I wrote the manuscript, with comments and edits provided by Dave and two anonymous reviewers.

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"Today [Banks Peninsula's] native biodiversity is sadly diminished. Nevertheless, when I completed my 1983–1988 detailed botanical survey of Banks Peninsula, my regrets about eagles and parakeets and forests were tempered by a realization of how much biological wealth still remained. An extraordinary diversity of living things had survived all the vicissitudes of the last few centuries – the burning, the felling, the hunting, the introduction of grazing and predatory mammals, the invasion by avian, insect and plant competitors contending with the original inhabitants for food and living space. Despite everything, I thought, the Peninsula was still a richly diverse, extraordinarily beautiful and unique place."

Hugh Wilson, 2002

Chapter 1

Introduction and study species

Forest fragment bordered by two types of exotic matrices, gorse and pine plantation, Tai Tapu.

1.1 Seed dispersal

With their sessile nature, plants are dependent on gene flow via seed to spread across the landscape. The spatial pattern of seed dispersal forms the base for subsequent demographic processes, including competition, predation and future reproduction (Nathan and Muller-Landau, 2000; Santamaría et al., 2007). At landscape scales, seed dispersal sets the parameters for colonisation of new sites and will influence probabilities of local extinction (Ouborg et al., 1999). All else being equal, seed dispersal will contribute twice as much genetic material as pollen dispersal, since seeds are diploid (both maternal and paternal genes) while pollen is haploid (paternal genes) (Hamilton, 1999).

Three non mutually exclusive benefits to seed dispersal are recognised: escape from disproportionately high mortality rates beneath the parent plant (Janzen, 1970; Connell, 1971), colonisation of sites favourable for germination (Howe and Smallwood, 1982; Wenny, 2001) and genetic mixing between populations. Additionally, directed dispersal suggests that seeds arrive in predictable locations that afford higher survival, but such dispersal is presumed to be rare (but see Wenny and Levey, 1998; Purves and Dushoff, 2005; Green et al., 2008).

Seeds are dispersed by a variety of abiotic (e.g. wind, water, gravity) and biotic (i.e. via animals) means. Biotic dispersal can be internal via consumption (endozoochory) or external by attachment (epizoochory); some seeds may be carried away with the intention of later consumption (e.g. scatter-hoarding by rodents). Where seeds are dispersed internally by animals, a mutualism is formed between the plant and animal; the plant provides a food source for the animal, with its seeds hopefully digested incidentally, and the animal later defecates or spits the seeds out. This mutualistic arrangement is more prone to cheating than pollination. Where pollination has a distinct target, i.e. the carpel of a conspecific flower, suitable germination locations will vary in space and time. Additionally, flowers have the benefit of providing incentive at the target site (pollen or nectar), while fruiting plants are burdened with the cost of "advance payment" and no such end point incentive. Seeds can also be a heavy luggage and dispersers, particularly volant animals, will certainly be inclined to dispose of this "ballast" as soon as possible (Wheelwright and Orians, 1982; Sorensen, 1984; Levey and Grajal, 1991; Stanley and Lill, 2002).

It is assumed for these reasons we do not see evidence of tightly co-evolved mutualisms between a single fruiting plant and its disperser (Wheelwright and Orians, 1982; Howe, 1993). Rather, within a given system one frugivore feeds on many plant species, each fleshy-fruited plant species is fed on by multiple frugivores, and the result is a "diffuse" mutualism (Zamora,

2000; Yoshikawa and Isagi, 2011). In the few cases where a tight relationship has been found between plant and frugivore, it is generally a case of large seed size or frugivore impoverishment (Loiselle et al., 2007). For instance, extinction of large-bodied frugivores has left *Laurus nobilis* in southern Spain almost entirely reliant on blackbirds (*Turdus merula*) for dispersal; in one study of fruit removal, blackbirds accounted for 96.6% of feeding observations to fruiting *L. nobilis* trees (Hampe, 2003). In another example, with the depauperate avifauna of the Yucatan Peninsula the tree *Bursera simaruba* is almost solely dispersed by a single avian species, *Vireo griseus* (Greenberg et al., 1995).

Several conditions are required for mutualism failure to have consequences for plant populations: native visitors do not provide the services required and exotic animals have not filled the gap, seed production is dependent on mutualists, and the plant's reproduction is dependent on seed production (Bond, 1994). The diffuse nature of dispersal mutualisms suggests there ought to be a level of redundancy (i.e. functional equivalence) amongst dispersal agents, perhaps buffering against the possibility of such mutualism failure (Zamora, 2000; Loiselle et al., 2007). Additionally, because only a subset of flowers produce fruits, the magnitude of dispersal required will be lower than that for pollination, suggesting lower densities of frugivores may be sufficient to provide adequate service (Kelly et al., 2004).

We cannot assume that loss of a dispersal mutualism will have an effect on plant populations, although this assumption is often made. The most popular example is the extinction of dodos (*Raphus cucullatus*) on Mauritius. These large ($\geq$ 12 kg), flightless birds were presumably the only disperser of *Sideroxylon grandiflorum*, a large-fruited (c. 5 cm diameter) tree endemic to Mauritius (Temple, 1977). Temple (1977) posited that the dodo and *S. grandiflorum* formed an obligate mutualism, such that *S. grandiflorum* now fails to regenerate in the absence of its disperser. Although this has since been refuted (Owadally, 1979; Witmer and Cheke, 1991; Cheke and Hume, 2008) the idea of an obligate mutualism failing after extinction of one of its participants has persisted and is still occasionally referred to in the literature (e.g. Bock, 2003; Katovai et al., 2012). Such an effect of mutualism failure is contingent on seeds requiring gut passage through an animal, and consequent removal of flesh, in order to germinate. Two benefits of such gut treatment are posited: deinhibition (removal of flesh releases seed from mechanisms inhibiting germination) and scarification (physical abrasion of the seed coat during digestion) (Traveset et al., 2007). Germination experiments comparing "clean" seeds (i.e. seeds which have passed through an animal's gut) versus those in intact flesh often find increased germination in clean seeds and conclude that this is a benefit of ingestion (e.g. Burrows, 1999). However, as fruit flesh will rot or leach off in normal field conditions, it has been argued that such experiments

do not accurately test the result of a loss of disperser (Traveset et al., 2007). Rather, germination experiments which allow for comparison of animal dispersed versus hand-cleaned seeds (to account for scarification of the seed coat via digestion) or allowing flesh to leach off under natural conditions, find that digestion by animals does not substantially improve germination (Izhaki and Safriel, 1990; Barnea et al., 1991; Traveset, 1998; Samuels and Levey, 2005; Robertson et al., 2006). Even if germination is not contingent on dispersal, failure of dispersal mutualisms may result in reduced removal of fruits, clumping of seeds beneath parents and increased density dependent effects such as seed predation (Loiselle et al., 2007).

Plants are unable to restrict what animals swallow their fruit, with the exception of increasing seed size. This is in stark contrast to the sometimes elaborate schemes of flower evolution to facilitate pollinator specialisation; think of orchids in the genus *Ophrys* with flowers resembling a female insect and males inadvertently picking up pollen while trying to mate (Raven et al., 1999). The "open" syndrome of fruits may allow naïve frugivores (e.g. exotic animals) to act as dispersers (Kelly et al., 2004); if this is the case, even extensive losses of native fauna may not result in mutualism failure. If exotic frugivores have been present long enough, their interactions with native plants may be important in sustaining ecosystem function (Zavaleta et al., 2001).

There are some clear instances in which invasive dispersers provide lower quality service for native plants. Where Argentine ants (*Linepithema humile*) have displaced native ants, dispersal distances of native seeds are reduced (Rowles and O'Dowd, 2009). This is due to a strong relationship between ant size and dispersal distance; invasive ants such as *L. humile* are typically smaller than native ant species and therefore disperse seeds shorter distances (Ness et al., 2004). Likewise, on many Pacific islands the native pigeons (Columbidae) have been more or less replaced by smaller frugivores, but these smaller birds appear to be deficient as dispersal agents (Chimera and Drake, 2010; Staddon et al., 2010). In other cases, island floras appear to be reliant on introduced frugivores for dispersal where the native island fauna is depauperate. On the Bonin Islands, off the coast of Japan, the introduced *Zosterops japonicus* appears to substitute at least partially for the less abundant, native *Apalopteron familiare*. Similarly, in Hawaii two introduced birds, *Z. japonicus* and *Leiothrix lutea*, are the most common frugivorous birds and appear to be responsible for the majority of dispersal service to native plants (Foster and Robinson, 2007). On New Zealand's Three Kings Islands, exotic birds may have sustained native plant dispersal in the absence of large-bodied native birds (Bellingham et al., 2010).

Where these exotic, or naïve, frugivores may fail is as "effective" dispersers. Schupp (1993) defined two aspects of disperser effectiveness: the quantity component refers to the numerical contribution of a frugivore and covers frequency of visitation, number of fruits removed and

frugivore abundance; the quality component concerns treatment of seeds (whether seeds are destroyed during feeding or digestion) and patterns of deposition. The typical disperser, according to Schupp, is deficient in one way or another; for instance, a disperser may consume large numbers of seeds but deposit them in areas unsuitable for germination.

Therefore, how a given bird or mammal effects seed dispersal depends on several factors: their abundance and distribution; the size of fruits they are able to swallow; their treatment of seeds (either as dispersers or predators); and the frequency of fruit in their diet (Levey, 1987; Schupp, 1993; Schupp et al., 2010). Factors affecting seed deposition are also important in dispersal effectiveness, including habitat use, feeding behaviour and movement patterns (Pratt and Stiles, 1983; Wheelwright, 1991; Alcantara et al., 2000; Westcott et al., 2005; Russo et al., 2006; Jordano et al., 2007; Martínez et al., 2008).

Although seed dispersal research has often focused on avian dispersers, a range of mammals which include fruit in their diet have been found to act as dispersers, including primates (Bleher and Böhning-Gaese, 2001; Wehncke et al., 2003; Martins, 2005; McConkey and Chivers, 2007), bats or flying foxes (Galindo-Gonzalez et al., 2000; Godinez-Alvarez et al., 2002; Nyhagen et al., 2005; McConkey and Drake, 2007), ungulates (Fragoso, 1997; Desbiez et al., 2009) and marsupials (García et al., 2009). Additionally, mammals whose diet does not typically focus on fruits can act as occasional dispersers, including carnivores (Pigozzi, 1992; Auger et al., 2002; Schaumann and Heinken, 2002; Jordano et al., 2007; Zhou et al., 2008) and herbivores (Davis et al., 2009).

For avian frugivores, fruit handling can affect the probability of seeds being moved away from the parent; as described above, internal dispersal of seeds presents a cost to the frugivore of carrying ballast, so there is an incentive to cheat, e.g. by consuming the flesh and spitting out the seed, which will generally result in seeds being dropped beneath the parent tree (Levey, 1987). Birds may alter their feeding behaviour to enable them to feed on larger seeds, including regurgitating large seeds (McConkey et al., 2004) or pecking the flesh of large fruits (Rey and Gutierrez, 1996).

Time spent in the fruiting tree will also influence whether seeds are moved away; the longer a frugivore spends in a tree after feeding, the more likely it is that seeds will be defecated while the bird is still in the parent tree (Pratt and Stiles, 1983; Wheelwright, 1991). There is some evidence that time spent in a tree is related to bird size; smaller birds may make shorter visits to fruiting trees due to predation risk or competition from larger birds (Pratt and Stiles, 1983). Frugivore density may also affect time in tree; in Tonga, McConkey and Drake (2006) found competitive interactions between flying foxes (*Pteropus tonganus*) reduced time spent in an individual tree. When flying fox abundance dropped below a certain threshold, interactions became less com-

mon and animals were more likely to defend a single tree and drop fruits beneath it. Because *P. tonganus* spits out seeds larger than 4 mm, when competition is high animals snatch fruits and fly away to consume them elsewhere. Similar processes may operate in plants dispersed by macaques, a species in which dominant males may drive subordinate individuals away from fruiting trees, inadvertently increasing seed movement away from parent trees (McConkey and Brockelman, 2011).

Differential movement patterns of dispersers is another source of variation. Spiegel and Nathan (2007) found in Israel that although two bird species dispersed similar quantities of seeds, their distinctive movements meant that *Onychognathus tristramii* dispersed most seeds over 600 m, while *Pycnonotus xanthopygos* dispersed most seeds within 500 m. In northern Spain, *Turdus* species tended to disperse seeds to covered microhabitats, while mammals (foxes, *Vulpes vulpes*, and badgers, *Meles meles*) deposited seeds in open areas, perhaps resulting in differential germination or predation based on characteristics of these microhabitats (Martínez et al., 2008). Similar results have been found in southern Spain where frugivorous birds were the major dispersers but generally dispersed over short distances, while foxes, badgers and stone martens (*Martes foina*) dispersed seeds over hundreds of metres (Jordano et al., 2007).

Given that the number of seeds handled by a frugivore is a function of the number of fruits per visit and the number of visits, an infrequent visitor that removes a large number of fruits per visit could prove to be an effective disperser (Fedriani and Delibes, 2009); this is particularly evident when those infrequent visitors make larger movements and thus may provide disproportionate long-distance dispersal relative to their feeding frequency (Jordano et al., 2007).

Although I have only discussed a few of the variations in disperser effectiveness, these variations suggest there will most certainly be differences between species in the quality of dispersal they provide, and also contextual differences within the same species. It may be that not only are seed dispersal mutualisms diffuse, but plants may benefit by having a range of species dispersing their seeds with each species performing different roles as predicted by Wheelwright and Orians (1982) and Wenny (2001) and perhaps confirmed in recent studies (Jordano et al., 2007; Spiegel and Nathan, 2007).

1.2 Measuring dispersal

The spatial distribution of seeds throughout the landscape is generated by tree locations plus the movements and behaviours of dispersal agents. Though there may be clusterings of seeds under bird perches, sleeping sites or feeding areas, locating dispersed seeds is generally only

possible through systematic sampling such as seed trapping or ground plots. However, once a seed is located in the landscape we do not immediately know which plant produced the seed, and thus do not know how far that seed has been dispersed.

The spatial distribution of seed dispersal is described by dispersal kernels, functions which describe the probability of seeds being deposited at varying distances from the parent plant (Nathan and Muller-Landau, 2000; Morales and Carlo, 2006). Dispersal kernels in nature are found to be largely leptokurtic, with a peak close to the source (i.e. the parent plant) and a long tail (Morales and Carlo, 2006); seeds in the tail are, by definition, less common than those near the mode (Portnoy and Willson, 1993). Fat-tailed distributions, where the kernel decreases slower than an exponential distribution (Clark, 1998), are increasingly being found with improved techniques for measuring accurate dispersal (Godoy and Jordano, 2001; Jones et al., 2005; Hardesty et al., 2006; Bittencourt and Sebbenn, 2007; Terakawa et al., 2008; Iwaizumi et al., 2010).

Accurate measurement of these kernels requires knowing both the source of seeds, and the location of their final destination; however, prior to molecular analyses few techniques were efficient at identifying both ends (Ashley, 2010). Direct observations on fruiting plants provide information on the source but no information regarding dispersal location, while seed trapping of dispersed seeds gives final location but does not immediately allow for identification of the source tree. Additionally, seed traps have limitations in their use for particular situations; they tend to be inefficient measures when seeds are deposited in non-random ways, for example when dispersers deposit seeds in clumps (Martínez et al., 2008). Seed traps also prevent possible secondary dispersal by alternate dispersal agents (e.g. scatter-hoarding rodents); in some cases, these secondary dispersers can move seeds to locations more favourable for germination (Vander Wall et al., 2005). Finally, seed trap methodology tends to assume the nearest conspecific tree is the parent (Nathan and Muller-Landau, 2000); DNA analysis of seed dispersal shows that a substantial minority of seeds do not come from this tree, so the "nearest-female" model under-estimates dispersal of these seeds (e.g. Hardesty et al., 2006; García et al., 2007).

Tracking seeds from the maternal parent (i.e. forward-tracking, Robledo-Arnuncio and García, 2007) has been trialled as a potential method, including the use of threads, tags, magnets, fluorescent dyes and radioactive and stable isotopes (Ashley, 2010). However, it has been estimated in oaks that tens of thousands of seeds or more would need to be tagged in order to achieve adequate sample sizes after seed predation reduces the available pool (Dow and Ashley, 1996). Animal displacement models, which infer potential dispersal patterns based on frugivore movement and habitat preference, have proven useful for modelling dispersal driven by animal dispersers. Studies have inferred seed rain by monitoring post-foraging movements of frugivores

(Spiegel and Nathan, 2007; Martínez et al., 2008) and by tracking animal movement (usually via radio-tracking) and comparing movement patterns to the gut passage of seeds (Murray, 1988; Sun et al., 1997; Holbrook and Smith, 2000; Westcott et al., 2005; Santamaría et al., 2007; Ward and Paton, 2007). The latter method has been utilised in New Zealand (O'Connor, 2006; Wotton, 2007). These models only measure theoretical dispersal, and do not analyse actual dispersal events. I discuss molecular methods for studying dispersal in Chapter 2.

1.3 Fragmentation

In addition to extinctions and extirpations reducing the animal species available to provide dispersal services, extensive habitat loss results in highly fragmented habitats that may also contribute to deterioration of mutualisms. Forest fragmentation simultaneously reduces the size of each population, increases isolation and, if species are unable to colonise the intervening matrix, reduces the availability of colonisation sites (Bacles et al., 2004).

Factors that may affect connectivity of fragments include isolation, fragment and population size (i.e. source strength), vegetative connections (e.g. corridors), dispersal mechanisms and seed sizes (Hewitt and Kellman, 2002). Repeatedly, findings suggest bird-dispersed seeds cross between fragments more frequently than wind dispersed seeds; wind dispersal, despite achieving long distances, distributes seed randomly downwind whereas birds may direct seeds to other fragments (Hewitt and Kellman, 2002). In some cases, frugivory may be enhanced in fragmented habitats, perhaps due to general impoverishment of food supply leading to a focus on that which remains (Farwig et al., 2006), though there is more evidence for lowered dispersal service in fragments, particularly due to reduced numbers of dispersal agents (Cordeiro and Howe, 2003; Rodriguez-Cabal et al., 2007; Kirika et al., 2008).

The typical view of habitat fragmentation has stemmed from Island Biogeographic Theory (MacArthur and Wilson, 1967), creating a dichotomy of suitable habitat ("islands") surrounded by an inhospitable matrix ("ocean"). This view is increasingly being challenged, the argument being that this "remnants as islands" view does not allow for the continuum of habitat suitability created by habitat loss and fragmentation (Fahrig, 2003; Kupfer et al., 2006). Current thought now leans toward the characteristics of the surrounding matrix aiding to determine the impacts of fragmentation. This matrix may provide alternative habitat, safe sites that encourage movement between fragments, or buffers against edge effects (Herrera and Garcia, 2009; Prevedello and Vieira, 2009; Herrera et al., 2011). Additionally, it is important to recognise that our perception of fragmentation is coloured by our inherent human biases; how a given species responds

to fragmentation is determined by the scale over which it operates. Where one species perceives a habitat as fragmented, for instance poorly dispersed plants or animals and insects with low mobility, other species may be able to move among fragments so easily that the landscape is essentially continuous (McIntyre and Hobbs, 1999).

Genetic consequences of fragmentation

Predictions regarding the genetic consequences of habitat fragmentation focus on reduced size and isolation. In theory, a reduced population size should lead to an erosion of genetic variation through the effects of genetic drift and inbreeding; these effects will be exacerbated by isolation which reduces or prevents gene flow (Templeton et al., 1990; Young et al., 1996). Such loss in genetic variation over time may limit the ability of species to respond to changes in selection pressures, for instance due to environmental change (Young et al., 1996). Genetic theory predicts one migrant per generation will minimise loss of genetic variation in subpopulations (Mills and Allendorf, 1996).

Plants ought to be particularly susceptible to loss of variability through habitat fragmentation, their sessile nature preventing them from moving between fragments. However, evidence to support the above predictions has proved elusive (Kramer et al., 2008). Furthermore, studies in plants are complicated by the wide variety of reproductive systems, including asexual reproduction, storage of genetic material as seeds, and interactions with animal pollen or seed dispersal vectors that may also be affected by fragmentation (Young et al., 1996). Large-statured plants may also be more affected by inbreeding; because plants do not have a segregated germ line, the higher number of mitoses over the life-span of large plants may increase the accumulation of deleterious mutations which can then be incorporated into gametes (Scofield and Schultz, 2006).

The addition of molecular analyses to studies of pollen and seed dispersal appears to be providing answers to historic paradoxes. Reid's Paradox (Reid, 1899; Clark et al., 1998) is based on the observations of Clement Reid, who noted that oaks colonising their contemporary British distribution after glaciations, required great dispersal distances in a short time frame. This seems paradoxical when we stand under an oak tree and observe the excessive number of acorns lying on the ground, apparently undispersed (Jones, 2010). Furthermore, Slatkin's Paradox (Slatkin, 1987; Mallet, 2001) observes that direct estimates of gene flow frequently underestimate the level of gene flow produced via indirect estimates, such as Wright's F_{ST}. Slatkin (1987) suggested the agreement, or lack thereof, between direct and indirect estimates of gene flow provides a indication of the importance of rare dispersal events in the population structure of a species. How-

ever, molecular analysis of dispersal distance, particularly via pollen, suggests that there may be no paradox at all; instead, it seems our previous "direct measures" of gene flow consistently underestimated Slatkin's "rare" events (Ashley, 2010). What we are beginning to realise is that until we know what we are not seeing, long-distance dispersal will seem rare (Jones, 2010). For instance, microsatellite studies of wind-dispersed pollen suggest upward of 20% of individuals sampled have a pollen parent outside of the study area, i.e, "immigrant" pollen (Ashley, 2010). No previous method could predict such high levels of long-distance dispersal. Similar long-distance movement of seeds (e.g. Jones et al., 2005; Hardesty et al., 2006; García et al., 2007) can easily explain Reid's Paradox too, allowing for seeds to colonise new areas rapidly (and if wind-pollinated possibly still be able to cross-pollinate with the parent population). The discovery of cryptic refugia will likely also go toward showing that there was perhaps never a paradox for at least some species (Provan and Bennett, 2008). Molecular methods may also aid in explaining why genetic erosion is not immediately evident in fragmented populations, as discussed above (Kramer et al., 2008).

1.4 New Zealand frugivores

Prior to European arrival, there was a suite of small- to mid-sized, volant avian frugivores most likely providing the bulk of dispersal service to native, fleshy-fruited plants (Atkinson and Millener, 1991). We have little diet information on birds that were extinct before Europeans arrived, and while moa were certainly large enough to swallow fruits of all native plants, it is unclear to what extent they did so. Other large birds eat fruit occasionally (e.g. kiwi, *Apteryx*) but do not appear to be major dispersal agents (Clout and Hay, 1989). Additionally, some dispersal by two Microchiropteran bat species may have occurred (Lord et al., 2002).

Of the extinct birds, huia (*Heteralocha acutirostris*, 300 g) and piopio (*Turnagra capensis*, c. 90 g) included fruit in their diet (Tennyson and Martinson, 2006). Huia appear to have largely fed on grubs and other insects, but was also recorded feeding on the fruits of plants such as *Elaeocarpus dentatus*, *Coprosma robusta* and *Rhopalostylis sapida* (Buller, 1888; Orbell, 2003). Buller (1888) found *E. dentatus* berries, along with arthropod remains, in the stomach of birds he examined. With a relatively large gape compared to other endemic birds (15 mm, Clout and Hay, 1989) huia were probably able to disperse larger fruits. Piopio had a smaller gape (11 mm, Clout and Hay, 1989), but were also recorded to feed on insects, worms and berries (Buller, 1888).

Several bird species have restricted ranges across New Zealand, but may have been important dispersers prior to human arrival: kokako (*Calleas cinerea*, 230 g), saddleback (*Philesturnus*

carunculatus, 70–80 g) and stitchbird (*Notiomystis cincta*, 30–40 g). Two subspecies of kokako are recognised: the North Island subspecies (*C. cinerea wilsoni*) is extant, with some small populations in heavily protected forests in the North Island (Innes et al., 1999); the South Island subspecies (*C. cinerea cinerea*) has not been sighted since 1967, and was declared extinct in 2004 (Tennyson and Martinson, 2006). The North Island kokako has been observed feeding on a variety of plant material. In Puketi State Forest, fruit made up 46–52% of the diet from summer to winter; annually, fruit comprised 44% of the diet, the highest of any one food type (Powlesland, 1987). In Rotoehu, fruits of four native species (*Hedycarya arborea*, *Myrsine australis*, *Litsea calicaris* and *Ripogonum scandens*) made up 21% of feeding observations in one year (Innes et al., 1996). Saddleback are mostly restricted to offshore islands, though they have recently been re-released into "Mainland Islands" (fenced reserves on mainland New Zealand with intensive pest control) (Heather and Robertson, 1996; Hooson and Jamieson, 2003; Armstrong and Davidson, 2006). On Motuara Island, translocated South Island saddlebacks fed predominantly on insects but also included honeydew, *Phormium cookianum* nectar, and *Pseudopanax arboreus* fruits (Pierre, 2000). Merton (1966) observed a female saddleback on Hen Island feeding on a *Corynocarpus laevigatus* fruit "parrot fashion", holding the fruit with one foot and ripping the flesh off before dropping the seed. On Hen Island, fruit made up 1–14% of the feeding observations depending on the time of year (Atkinson, 1964, 1966; Merton, 1966). Stitchbirds are the smallest of New Zealand's honeyeaters and are restricted mostly to offshore islands. They have been observed feeding on a variety of fruiting species on Little Barrier Island, including *Myrsine australis, Macropiper excelsum* and *Geniostoma ligustrifolium* (Gaze and Fitzgerald, 1982).

Three endemic frugivores are still abundant; kereru (*Hemiphaga novaeseelandiae*, 650 g), tui (*Prosthemadera novaeseelandiae*, 90–120 g) and bellbird (*Anthornis melanura*, 26–34 g). Additionally, the naturalised silvereye (*Zosterops lateralis*, 13 g) consumes fruits, though it is limited by its small gape. All four species feed on a range of native fruits, with fruit diameter and gape size apparently the only limitation to what fruits they will take (O'Donnell and Dilks, 1994; Williams and Karl, 1996; Emeny et al., 2009; Kelly et al., 2010).

Avian introductions

During the period of intense introduction of exotic species, particularly by acclimatisation societies, 42 species of passeriform birds were introduced to New Zealand; of these, 15 established successfully in at least one of the four acclimatisation districts (Auckland, Canterbury, Otago and Wellington) (Duncan, 1997). Of these species, three are potential dispersers of native fruits, due to their function as dispersers in their native range: European blackbird (*Turdus merula*, 90

g), song thrush (*Turdus philomelos*, 70 g), and European starling (*Sturnus vulgaris*, 85 g) (Herrera and Jordano, 1981; Snow and Snow, 1988; Debussche and Isenmann, 1989; Rey et al., 1997; Fuentes et al., 2001; Hernandez, 2005; Linz et al., 2007). All three species are abundant and appear to function well in New Zealand, possibly due to lack of competition, absence of ectoparasites, or climatic variables (Duncan, 1997; MacLeod et al., 2009).

1.5 Current status of dispersal in New Zealand

Clout and Hay (1989) concluded, given the gape sizes of extant native and introduced birds, that six plant species appear almost solely reliant on kereru for dispersal. However, analysis of fruit shape across the New Zealand flora suggests there is a tendency for fruits to become elliptical as they get bigger, thereby reducing seed diameter proportionate to overall seed mass (Lord et al., 2002). As kereru have a distensible bill, they are able to swallow all native fruits regardless of size; therefore, for this trend in fruit shape to occur there must have been a selective pressure for dispersal by mid-sized birds (Kelly et al., 2010).

A more recent analysis, with additional feeding data, has shown that native birds can feed on fruits larger than that predicted from discrete values of gape width and fruit diameter. Kelly et al. (2010) theorise that natural variation of fruit size or gape size within a given species allows this expansion of feeding data. As an example, tui have a measured mean gape of 9.7 mm, but a maximum of 11 mm; this would allow a larger gaped tui to swallow fruits above 10 mm diameter. Similarly, fruit sizes vary about their mean in such a way that smaller individual fruits may be swallowed by birds that according to the means given by Clout and Hay (1989) would be too small to swallow that particular species (Kelly et al., 2010). This now reduces the six species predicted to rely largely on kereru for dispersal (Clout and Hay, 1989) to a single species (*Beilschmiedia tarairi*) for which kereru truly are the only native disperser (Kelly et al., 2010).

Therefore, in New Zealand there is a range of avian species, both endemic and exotic, that are able to consume a wide range of native fruits. As I have discussed, there may be benefits to plants by having an assemblage of dispersers feeding on their fruits, in particular as redundancy among dispersers may buffer against mutualism failure. Additionally, introduced mammals may swallow fruits, and larger mammals will be less affected by large fruit size, but their role in New Zealand has been given little attention until recently.

1.6 Study species: matai (*Prumnopitys taxifolia*)

To elucidate the role of the contemporary suite of avian dispersers in the movement of native seeds, I chose to study matai for several reasons. It has an intermediate-sized fruit (mean diameter = 9 mm) which is swallowed by a range of native and introduced birds including bellbird, blackbird, song thrush and tui (Kelly et al., 2010). Studying one of these intermediate-sized species allows questions regarding the suite of dispersers, rather than focusing on the few cases of reliance on large dispersers. Additionally, because matai is long-lived (trees can live to over 1000 years, Lusk and Ogden, 1992) adults remaining after deforestation may retain genetic diversity presenting an extinction debt (Tilman et al., 1994).

Matai is a New Zealand endemic emergent canopy tree in the family Podocarpaceae. Worldwide there are 8–10 species recognised in the genus *Prumnopitys*, two of which are endemic to New Zealand (*P. taxifolia* and miro, *P. ferruginea*). Suggested revisions have been made that would split the genus into at least two groups. These two groups are largely based on the male and female reproductive structures which can be spicate (e.g. *P. taxifolia*, see Chapter 2 frontispiece) or solitary (e.g. *P. ferruginea*) (Salter, 2004).

Matai has drupaceous fruits, roughly circular with a single enclosed seed (mean diameter = 7 mm). As an adult, matai can grow to 25 m or more, with a trunk reaching 1.25 m diameter. At this height it is a canopy emergent, with several metres of its canopy extending above the surrounding broadleaf canopy. Matai is a heteroblastic species, with juveniles forming a divaricating shrub with different leaf shape and branching pattern to that seen in adults (see Chapter 6, Figure 6.1 for an example) (Philipson and Molloy, 1990); this juvenile phase possibly persists for over 20 years. At a height of 4–6 m and trunk diameter of approximately 10 cm, there is a gradual transition to adult form, although it is unclear when trees reach reproductive maturity (Salter, 2004). One analysis of stems estimated that matai took 60 years to grow to 3 metres in height, the longest period of the four podocarp species studied (Beveridge, 1973). Matai has consistently shown slower seedling growth than the other four studied New Zealand podocarp species, both in the field and in glasshouse conditions (Beveridge, 1973; Ebbett and Ogden, 1998).

Development of reproductive structures stretches over two years. Male strobili appear in summer, overwinter and then shed their pollen the following spring/summer. Ovules appear in spring shortly before pollen is released. Unripe seeds remain the same size over winter and grow to full size the following summer, ripening in late summer to early autumn (April/May). Thus, fruits are available approximately 14–16 months following pollination (Leathwick, 1984; Salter, 2004). Females produce seeds on spicate structures that can hold up to 12 fruits (Sullivan et al.,

1995). There are some observations that suggest individual trees may show some form of leaky dioecy (see Chapter 3 for further discussion).

Matai seeds have a thick coat (see Chapter 2 for anatomical details) which has been posited as an anachronism developed for dispersal by moa (Thorsen et al., 2009; Kelly et al., 2010); such anachronisms have been described elsewhere where fruit or seed traits suggest adaptation for dispersal by extinct species (Janzen and Martin, 1982; Guimarães et al., 2008). However, this assertion with regard to matai has not addressed that this thick seed coat is also found in *P. ferruginea* and other *Prumnopitys* outside of New Zealand (e.g. *P. andina*, Mill et al., 2004).

Whereas some other podocarps (e.g. kahikatea, *Dacrycarpus dacrydioides*, and rimu, *Dacrydium cupressinum*) show a marked periodicity in their fruiting (Norton and Kelly, 1988), matai may be more driven by failure of the fruit crop in some years. In a North Island forest, matai failed to produce a good seed crop over seven years; one possibility is that much of the seed crop in a given year is attacked by insect predators (Beveridge, 1973), although some seed trap evidence seems to correlate with the occurrence of drought (C. J. Burrows, unpub. data).

Matai and the other canopy podocarps are characterised by being late successionals, having juveniles tolerant of shade and root competition with slow growth to adulthood (Burrows, 2006). Burrows (2006) describes matai as being able to establish and grow slowly under dense canopy, having suppressed juvenile growth under canopy but growing vigorously in canopy gaps, and regenerating after disturbance. At heights of 1–2 m, podocarps produce small annual shoot growth (2–5 cm) which is offset by shoot death so that annual growth stagnates. However, if a canopy gap appears saplings can increase their mean annual growth up to 30 cm; kahikatea shows the greatest growth rate in these released conditions, while matai shows the slowest (Beveridge, 1973).

Old growth stands of podocarps, both in New Zealand and overseas (e.g. Chile, Tasmania), are characterised by a cohort of canopy or emergent trees with few saplings and poles present (Wardle, 1963; Lusk and Ogden, 1992). It is likely that these patterns are driven by the occurrence of rare disturbance events knocking out the canopy tier (Duncan, 1993; Wells et al., 1998, 2001; Cullen et al., 2003), followed by regeneration into canopy gaps then stagnation by species that are unable to regenerate beneath a closed canopy (Lusk and Ogden, 1992).

1.7 Study area: Banks Peninsula

I chose to work on Banks Peninsula because although there has been extensive deforestation throughout the history of human presence in New Zealand, there is still a network of old-growth

forest fragments across the peninsula, many of which have remnant podocarps including matai, kahikatea and totara (e.g. frontispiece, this chapter). The original forest cover was largely continuous podocarp-broadleaf forest, so the current forest fragments represent relatively recent fragmentation. It is unfortunate that tui are not present on Banks Peninsula, they are locally rare throughout Canterbury, as they certainly disperse matai in areas where both species co-occur (Kelly et al., 2010), but a range of native (bellbird, kereru) and introduced (blackbird, song thrush, starling) birds are present (Freeman, 1999; Deconchat et al., 2009; Spurr et al., 2011).

Kahikatea, matai, miro and totara (*Podocarpus totara*) were common across Canterbury prior to human arrival, as indicated by subfossil evidence from the area (Molloy et al., 1963; Molloy and Brown, 1995). Much of the forest on the Canterbury Plains was lost to fire soon after human arrival. The best estimate suggests Polynesian settlers arrived in New Zealand about 1280 AD. Although kiore (*Rattus exulans*) bones were reportedly dated to 2,000 years BP (Holdaway, 1996), these dates have been disputed and a re-dating of the bones found none older than 1280 AD (Wilmshurst et al., 2008). These dates also fit with archaeological evidence of human arrival and dating of seed cases gnawed by rats, all suggesting humans arrived in New Zealand in the 13th Century (Wilmshurst and Higham, 2004).

The eastern South Island lies within a rain shadow, creating dry forests vulnerable to fire. New Zealand's maritime climate means that convection-induced thunderstorms are uncommon (except in the interior mountains, where fire risk is generally low), so prior to human arrival there was little in the way of ignition sources (Ogden et al., 1998). While in the North Island forest loss due to fire was strongly linked to surrogates for human presence (e.g. proximity to lakes), such a relationship is not apparent in the South Island. This decoupling of fire patterns and patterns of human habitation may indicate that human arrival in the South Island introduced an ignition source which then caused widespread fire (Perry et al., 2012*b*). This effected a transition from closed-canopy forest cover to shrub and grassland (McWethy et al., 2009, 2010); this replacement vegetation cover is more flammable than late-successional forest and so positive feedbacks may have occurred by which an increase in fire frequency results in an increase in flammable vegetation, which in turn increases average fire size (Perry et al., 2012*a*).

The "Torlesse map," produced in 1859 by Charles Torlesse for the Canterbury Association, gives the closest approximation to vegetation cover in Canterbury at European arrival. Excluding Banks Peninsula, native forest (beech or podocarp dominated) is shown on less than 5% of the Canterbury Plains. By 1885, timber harvest had reduced this forest cover to 12% of the extent at the time of the Torlesse map (i.e. 0.6% of the plains); almost no trace now remains of these plains forests (Pawson and Holland, 2008).

Banks Peninsula encompasses 100,000 ha, 50 km long by 30 km wide and rising to 920 m, based around two volcanic craters (Lyttelton and Akaroa) which were formed 9–11 MYA (Bradshaw and Soons, 2008; Wilson, 2008). The Port Hills sit on the western side of the Lyttelton crater, separating the city of Christchurch from the peninsula. The volcanic landmass was separated from the rest of the South Island, effectively existing as "Banks Island", until the formation of the Canterbury Plains. Glacial and inter-glacial cycles dominated New Zealand's climate over the past 800,000 years; with each ice advance, sediment was carried down from the Southern Alps by meltwater and spread as alluvial fans. By the late Pleistocene the advancing plains reached the western slopes of the Lyttelton crater, joining Banks Island to the rest of the South Island and forming Banks Peninsula (Bradshaw and Soons, 2008).

Prior to human arrival, Banks Peninsula was almost fully covered with forest; tall podocarps at lower elevations gave way to broadleaf forest higher up the slopes (Soons et al., 2002). Pollen records from the penultimate interglacial show a dominance of matai and other podocarps, growing along with understorey broadleaved species (Soons et al., 2002). All five of the main podocarps were present on Banks Peninsula; totara dominated on dry hillsides (lowland totara (*P. totara*) below 500 metres, and Hall's totara (*P. hallii*) above 500 m), with matai and kahikatea in abundance on swampy valley floors. Both these species also co-habited with totara on the hillslopes, but totara remained the dominant podocarp in these areas. Miro appears to have had a scattered distribution, and rimu was rare, being present only in a restricted area around Little River (Petrie, 1963).

The earliest evidence for settlement of Banks Peninsula, from radiocarbon dates of archaeological sites, date to 700 years BP. These first arrivals would have encountered a landscape almost fully covered by native forest. Over the next few centuries, about 30,000 hectares of old-growth forest was cleared by fire, whether accidental or deliberate. This represents a quarter to a third of the pre-human forest cover. Alongside this loss of forest, an estimated 29 bird species (from approximately 100 species) became locally extinct on the peninsula. Extinction of native plants, however, was probably low or non-existent (Burrows, 1994*a*; Wilson, 2004, 2008).

European arrival

> "I have heard it suggested as a great obstacle to the plains, the absence of wood. Bank's Peninsula alone would supply twenty Canterbury Settlements for centuries."
> - Chief Surveying Officer of H.M.S "Acheron". 8 May 1849.

The history of deforestation on Banks Peninsula is one of the best documented in New Zealand.

At the time of European arrival, approximately two-thirds of the peninsula was forested; within 60 years only 1.2% of the peninsula remained under forest (Wilson, 2004). Of the remaining forest remnants, the majority of "large" reserves ($>$ 40 ha) are situated on steep terrain, e.g. hilltops or valley heads (Wilson, 2004). Protection of forest remnants by landowners and encouragement of native vegetation regeneration means the peninsula is now regenerating to native cover across approximately 25% of its area (Wilson, 2004). Exotic forest covers approximately 2,000 ha, the majority of which is *Pinus radiata* plantation (Deconchat et al., 2009).

Prior to the establishment of Christchurch in 1850, there had been very little impact of European arrival on the peninsular forests; whalers' impacts were limited by their access to tools, while early settlers were mostly seeking grazing land and so targeted tussock country. The arrival of pit-sawing in 1849 signalled the start of what would be fifty years of intense deforestation; by 1860, the timber trade was dominated by sawmills, several of which operated up to the early 20th Century (Petrie, 1963; Ogilvie, 2007).

At Piper's Valley mill near Duvauchelle, totara was the main timber (90%) with additional Hall's totara, matai and kahikatea. Other mills probably harvested similar compositions, with rimu only harvested around Little River (Petrie, 1963). With the installation of steam engines, annual timber processing doubled; at Robinson's Bay the annual take was over one million feet (Petrie, 1963). Even such seemingly large harvests did not remove the entire forest cover; the sawyers and millers were primarily interested in "timber trees" with tall, unbranched trunks. However, as more settlers arrived and required land for farming the remaining forest represented a hindrance to grassland growth (Petrie, 1963). The tragic irony is that forested land is not necessarily the most fertile; such thoughts were based on the northern hemisphere theory that areas with the greatest biomass held soils with the greatest fertility (Wood, 2003). In fact, for New Zealand forests it appears that podocarps can only out-compete angiosperms in certain conditions, one of these being Phosphorus-depleted soils (Coomes et al., 2005; Carswell et al., 2007). If true, then removing podocarp forest for farming left behind rapidly deteriorating soils that were in fact inferior for agriculture.

I conducted the majority of my research across seven forest fragments on the Port Hills, Banks Peninsula (Figure 1.1, Table 1.1). All of the sites are old growth forest remnants with emergent adult podocarps present (kahikatea, matai, totara), sometimes in close proximity (see Chapter 3 frontispiece). Fencing regimes and levels of pest control vary across the sites (Table 1.1; see Appendix A for specific details). The seven sites I used were spaced along approximately 7 km of Summit Road, which runs along the top of the Port Hills, and ranged from the lower slopes of the hills (c. 40 m a.s.l) to the height of the road (c. 500 m a.s.l). These sites include

all remnants of old growth forest within the area, although there are further fragments on the eastern side of the hills (e.g. Living Springs in Figure 1.1). All of the sites are no more than 1.5 km from the next site (minimum 300 m, Figure 1.1) suggesting it will be possible for seeds to be dispersed between neighbouring fragments. Further information on each site, including mapped adult matai trees, is provided in Appendix A.

1.8 Thesis outline

The main objective of this thesis was to investigate the current dispersal status of matai in contemporary environments; that is, fragmented forests with a suite of both native and introduced dispersers. As matai fruits are intermediate sized, they are available to be consumed by a wide range of native and exotic birds, allowing study of the disperser assemblage. I examined several stages of dispersal of matai, including the first study to my knowledge to attempt to apply genetic analyses to dispersal of a New Zealand plant.

In Chapter 2 I investigate the feasibility of using microsatellites to genetically identify the source tree of dispersed seeds. To this end, I isolated microsatellite loci via high-throughput sequencing, developed primers to allow amplification of these loci, and investigated polymorphism across a sample of adult matai sourced from Banks Peninsula. I then attempted to apply these data to the measurement of seed dispersal of matai. I provide a thorough introduction to the process of microsatellite development and genotyping.

The remainder of this thesis focuses on field-collected data. In Chapter 3 I use seed traps and ground plots to measure the annual seed fall of matai on the Port Hills, and examine the influence of insect and rodent predators. I discuss the spatial movement of matai seeds and the proportion of seeds apparently dispersed by birds to assess whether dispersal service is provided in forest fragments. In Chapter 4 I present data on bird visitation to fruiting matai trees. There is some question regarding the various roles of native and introduced birds in seed dispersal of native fleshy-fruited plants (e.g. Kelly et al., 2006); therefore, by conducting long, sitting observations (cf. mist netting or walking transects) I aimed to elucidate the relative importance of native and introduced birds. I then discuss the relevance of my results to previous studies of New Zealand avian frugivory. Following from this, in Chapter 5 I investigate the possibility of feral pigs acting as seed dispersers of matai; thus, furthering the discussion of the roles of introduced animals in dispersal mutualisms. Finally, in Chapter 6 I synthesise my findings and discuss the implications for the status of dispersal in today's altered environment.

Table 1.1: Details of field sites on the Port Hills, Canterbury. References: Kelly, 1972; Burrows, 1986, 1994*b*; Wilson, 1994. CCC = Christchurch City Council; QEII = Queen Elizabeth II National Trust covenant.

Site	Easting/Northing (NZTM)	Altitude (m a.s.l)	Area (ha)	Tenure	Fencing
Ahuriri Summit (AS)	1569600, 5165094	400–470	10.9	CCC	Well fenced, some pest control
Ahuriri Valley (AV)	1567501, 5163194	40–200	80	QEII	Fenced, some stock incursion
Cass Peak (CP)	1569500, 5168292	c. 450	3.2	CCC	Fenced
Kennedys Bush (KB)	1569500, 5169092	290–490	86.5	CCC	Fenced, some goats
Omahu Bush (OM)	1568701, 5165393	200–470	60	QEII	Fenced, but low upkeep has resulted in stock incursion
Otahuna Reserve (OT)	1568301, 5167293	100–200	11	CCC	Fenced, but ungulate presence
Tai Tapu (TT)	1566402, 5164194	c. 250	7	Private	Unfenced, heavy ungulate presence

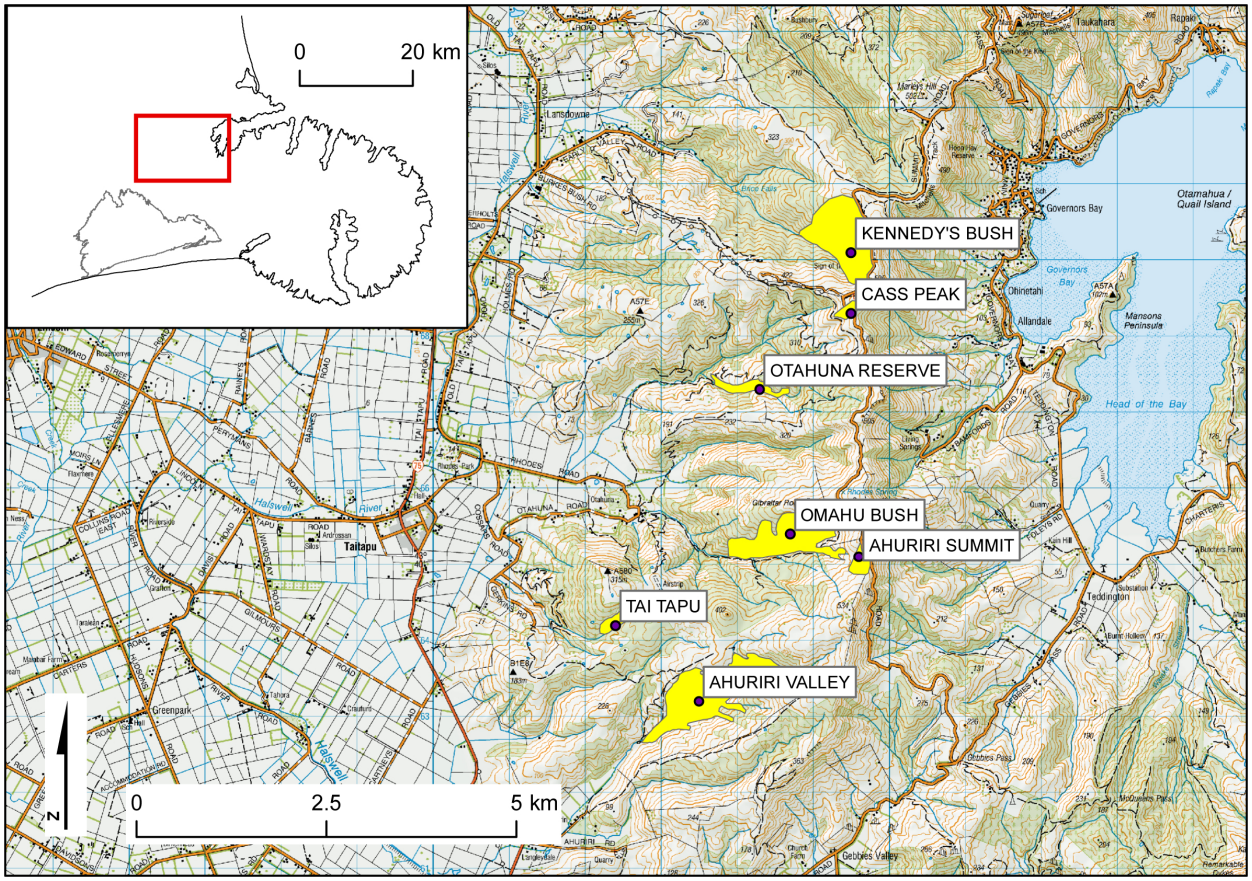


Figure 1.1: Location of field sites on the Port Hills

Chapter 2

Microsatellite development and individual identification of matai trees

Green matai fruits showing spicate structure.

2.1 Abstract

Molecular markers have been applied to the measurement of seed dispersal in response to the difficulty in determining both the origin and the final destination of dispersed seeds; of the range of markers applied, microsatellites (SSRs) are proving the most popular due to their high polymorphism and codominant inheritance. I used next-generation sequencing to search for microsatellite repeat motifs within the genome of matai (*Prumnopitys taxifolia*) and developed primers for 30 microsatellite loci. Of these, 16 amplified sufficiently for genotyping so I had one primer from each pair fluorescently labelled; although six loci did not amplify consistently enough to allow genotyping, I was able to genotype 21 adult matai (20 from Banks Peninsula, one from Kaikoura) across ten loci. Of these ten loci, five were polymorphic, two were functionally monomorphic (they exhibited a single rare allele), and three were monomorphic across all 21 trees. Among the 20 Banks Peninsula trees, none shared a multi-locus genotype suggesting that it will be possible to identify individuals using these five polymorphic loci. Probability of Identity analysis indicated these five loci would provide high exclusion probabilities for distinguishing individual trees. Seed coat extractions were largely contaminated (most likely with phenols and polysaccharides) which inhibited PCR amplification; time restrictions meant I could not resolve these issues. However, preliminary results suggest that it will be possible to identify maternal parents of matai seeds using these polymorphic microsatellites.

2.2 Introduction

Several molecular methods have been employed to measure gene flow, including mitochondrial haplotype differentiation (Johansen and Latta, 2003), AFLPs (Arens et al., 1998; He et al., 2004), allozymes (Bacles et al., 2004), and spatial structure of maternally inherited mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) (Latta et al., 1998; Oddou-Muratorio et al., 2001). Amplified fragment length polymorphisms (AFLPs) and allozymes have largely been replaced by microsatellites which have been applied to wind- (Ziegenhagen et al., 2003) and mammalian-dispersed seeds (Grivet et al., 2005; Pairon et al., 2006; Terakawa et al., 2008), pollen dispersal by wind (Dow and Ashley, 1996, 1998) and insects (Dawson et al., 1997) and comparing pollen and seed dispersal distances (Heuertz et al., 2003; Goto et al., 2006; Hardesty et al., 2006; Bittencourt and Sebbenn, 2007; Yu et al., 2010). The rising popularity of microsatellites is due to their high polymorphism and codominant inheritance, this allows both alleles in a heterozygote to be identified, compared with dominantly inherited markers in which homozygotes and heterozy-

gotes are indistinguishable (Lowe et al., 2004).

In Chapter 1 I discussed the difficulties in measuring dispersal via tracking seeds from their parent plant; in response to this problem, molecular techniques have been developed to aid in identifying the source of a dispersed seed. Two issues arise in determining the maternal origin of seeds via molecular analysis: first, in outcrossing cosexual species (monoecious or hermaphrodite) it is impossible to determine from embryonic tissue which parent provided the seed (Godoy and Jordano, 2001); second, if molecular markers are not variable enough to distinguish individuals, there will often be several potential parents and generally the closest will be selected as the probable parent (possibly under-estimating dispersal).

This first issue can be circumvented in two ways; first, studying dioecious species simplifies analysis because the search will be for a compatible male and female pair, second, by using tissues or organelles that are uniparentally inherited (Ashley, 2010). Organellar study is particularly interesting in conifers, as chloroplasts appear to be paternally inherited (Wagner, 1992; Lowe et al., 2004) and microsatellites have been isolated from cpDNA (Provan et al., 2001; Weising et al., 2005). However, preliminary work suggests organellar DNA is not variable enough for individual identification (Ashley, 2010).

This difficulty in distinguishing maternal and paternal parents led to two trends in the analysis of plant gene flow. First, there has been an emphasis on the study of pollen dispersal; this is because seeds can be collected directly from a maternal parent (thus the maternal parent's identity is already known) and paternal exclusion analysis used to identify potential pollen donors. Second, where seeds have been collected post-dispersal, therefore neither parent is known, and two parents are identified the closest is assumed to be the seed parent (e.g. Dow and Ashley, 1996). Thus, seed dispersal has been generally under-represented in molecular analysis of plant gene flow, and where it has been studied has likely been systematically under-estimated due to technical limitations.

Godoy and Jordano (2001) developed a new technique to allow more focus on measuring seed dispersal; using maternally derived tissues, in their case the seed endocarp, which exhibit the same genotype as the seed parent they successfully identified the maternal origin of dispersed seeds. This technique has been applied to other seed tissues, including pericarp (Grivet et al., 2005) and seed wings (Jones et al., 2005), so long as the tissues are maternally derived.

2.2.1 Suitability of matai

Matai is diploid (2n = 38); along with miro (*P. ferruginea*, 2n = 36), these two species have among the highest number of chromosomes among the podocarps (Davies et al., 1997). Polyploidy

complicates the analysis of microsatellite loci (Ashley, 2010), but appears to be rare in conifers (Ahuja, 2005).

For the method of Godoy and Jordano (2001) to work, the tissues used in seed coat extractions must have a maternal origin, and therefore express the same genotype as the seed parent. Gymnosperms exhibit a wide range of seed anatomies, and it is important to assess whether a species is suitable for maternal analyses. Gymnosperm seed coats typically comprise three layers: an outer parenchymatous layer (sarcotesta), a middle sclerenchymatous layer (sclerotesta), and an inner parenchymatous layer (endotesta). The endotesta generally collapses at maturity, leaving a thin membranous layer. All three tissues are derived from maternal tissue, and are therefore diploid (Bewley et al., 2006). Podocarpaceae have an additional layer, the epimatium, unique to the family that encloses the ovule and is thought to be homologous to the oviliferous scale (Mill et al., 2004; Salter, 2004). In arillate podocarps (e.g. *Dacrycarpus*, *Dacrydium* and *Podocarpus*) the epimatium is a thin layer enclosing or fused to the integument; however, in *Prumnopitys* the epimatium becomes swollen and fleshy (Salter, 2004). Therefore, in matai the sarcotesta is comprised of the outer integumentary layers and the epimatium fused together. The sclerotesta begins to form a hard, woody layer at fertilisation and is approximately 1 mm thick and very hard by the time of seed dispersal (Mill et al., 2004; Salter, 2004) Therefore, the sclerotesta may be considered analogous to the woody endocarp used in *Prunus mahaleb* by Godoy and Jordano (2001). It should exhibit the same genotype as the maternal parent, although it is prudent to confirm this via known parent-offspring comparisons. For ease of terminology and correlation with other studies, I refer to the woody sclerotesta as the "seed coat" and the sarcotesta as the fruit flesh. Clearly neither are botanically correct, but this will allow consistent terminology to other ecological studies of seed dispersal which have largely focused on angiosperms.

Additionally, gymnosperms are interesting for genetic analyses of seed dispersal because they retain a haploid megagametophyte, rather than being replaced by the triploid endosperms found in angiosperms as a result of double fertilisation. In gymnosperms the second sperm nucleus degenerates (Raven et al., 1999). It is important to note that the haploid megagametophyte is derived from the ovule (Bewley et al., 2006), therefore it will exhibit the same genotype (regardless of meiotic segregation) as the maternal contribution to the embryo. This anatomical peculiarity has recently been taken advantage of for determining biparentage identification; by genotyping the maternal, haploid megagametophyte and comparing this to the diploid embryo genotype, the maternal genotype can be excluded and the pollen donor identified with a higher likelihood than using embryonic tissue alone (Iwaizumi et al., 2007, 2010). Such genetic

and anatomical peculiarities in gymnosperms open a variety of avenues for research not available when studying angiosperms; within gymnosperms, members of Podocarpaceae, with their fleshy, animal-dispersed "fruits" are an ideal study species for animal-mediated dispersal.

2.2.2 Microsatellites

Microsatellites are repetitive DNA sequences of 1–6 bases; for example, an $(AT)_n$ microsatellite consists of the nucleotides adenine (A) and thymine (T) repeated n times. Microsatellites are present in both coding and noncoding regions, and exhibit high polymorphism in their length, i.e. the number of times the repeat occurs (Zane et al., 2002). Although typically assumed to be neutral to selection, there is some evidence that microsatellites may have functional roles and therefore be subject to selection (Li et al., 2002). They are assumed to be randomly distributed throughout the genome, including nuclear DNA (nDNA), mtDNA and cpDNA (Lowe et al., 2004); although originally isolated from mammalian genomes, microsatellites were also found to be ubiquitous in plant genomes (Morgante and Olivieri, 1993). The most frequent motifs found in plants are $(A)_n$, $(AT)_n$, $(GA)_n$ and $(GAA)_n$ (Weising et al., 2005).

There are some disadvantages to the use of microsatellites. Until recently, initial identification was expensive and time-consuming, and since flanking regions are generally species specific primer development is required for each new study species (Lowe et al., 2004). Additionally, because microsatellites appear to mutate in a step-wise fashion (i.e. each mutation adds or deletes one repeat) there is a high level of homoplasy between alleles. That is, alleles of the same size have arisen through different lineage but will appear identical; this lowers visible allelic diversity (but may be able to be assessed through direct sequencing of alleles) and may limit the usefulness of microsatellites for phylogenetic studies (Lowe et al., 2004; Selkoe and Toonen, 2006).

Traditionally microsatellites were isolated by cloning segments of DNA and probing for desired microsatellite sequences (Zane et al., 2002). The biggest drawback to this method is the necessity of creating genetically modified bacteria; particularly when working in New Zealand with native species, this step may be prohibitive in both cost and regulatory logistics. However, with the advent of next-generation sequencing (NGS), e.g. 454 sequencing (Roche, Penzberg, Germany), that allow high throughput sequencing, this cloning step is not required. Briefly, 454 sequencing involves breaking genomic DNA into fragments (300–800 bp), ligating adaptors to fragment ends and immobilising these fragments on to DNA capture beads. The beads are then exposed to Polymerase Chain Reaction (PCR) ingredients, turning each bead into a microreactor for PCR amplification; thus, each bead holds one fragment which is then loaded onto a

sequencing plate. The resulting data are a series of sequenced fragments spread throughout the genome (Ellegren, 2008; Abdelkrim et al., 2009). Because microsatellites are ubiquitous throughout eukaryote genomes (Lowe et al., 2004), generating random sequences of DNA should include some of these microsatellite repeats (Abdelkrim et al., 2009). The resulting sequence library can then be searched for repeat motifs, and primers developed based on the flanking sequences of these repeats.

Although there are several NGS technologies available, the Roche 454 technology is preferred for microsatellite development due to the larger average fragment sizes obtained through sequencing (200–300 bp); larger sized fragments will increase the probability of finding microsatellites with sufficient flanking regions on either side of the repeat motif to allow for primer design (Gardner et al., 2011). In fact, the Roche 454 is so preferred that in a recent review of 15 NGS studies, all of them used this system (Guichoux et al., 2011). NGS has been used successfully for isolating microsatellites from a range of species and tissues, including ancient DNA (Allentoft et al., 2009).

Some form of primer is necessary to provide the 3' hydroxyl end required for DNA polymerase to synthesise a complementary strand of DNA during PCR replication (van Pelt-Verkiul et al., 2008). Figure 2.1 illustrates the role of these primers: two primers are required, one for either flanking region, which allow preferential amplification of the intervening microsatellite (i.e. the target product). Primers are preferentially 18–22 bp to limit the chance of the primer binding at multiple places in the genome (van Pelt-Verkiul et al., 2008). The flanking regions on either side of microsatellite repeat motifs are assumed to be conserved across a species, allowing the design of specific oligonucleotides that anneal to these sequences (Lowe et al., 2004; Selkoe and Toonen, 2006).

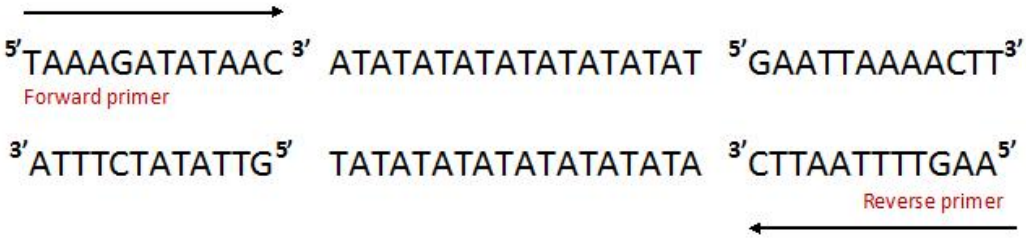


Figure 2.1: Schematic illustrating the purpose of oligonucleotide primers. Arrows indicate the direction of DNA replication (i.e. 5' to 3'). The microsatellite repeat (AT_9) in the centre is flanked by two regions. Primers are designed to fit to these flanking regions, thus providing the 3' hydroxyl end required for DNA polymerase. A pair of primers (forward, reverse) allows replication of the microsatellite motif in between.

2.2.3 Genetics of New Zealand plants

There have been several genetic studies of endemic New Zealand plants to date. Several different molecular markers have been used: allozymes (Billington, 1991; Haase, 1992*a*,*b*, 1993; Hawkins and Sweet, 1989), AFLPs (Broadhurst et al., 2008), random amplification of polymorphic DNA (RAPD) (Holzapfel et al., 2002) and more recently microsatellites (Barrell et al., 1997; Young et al., 2001; Shepherd et al., 2008). These genetic studies have ranged across both conifers (*Dacrydium cupressinum*, Hawkins and Sweet, 1989; *Halocarpus bidwillii*, Billington, 1991) and angiosperms (*Nothofagus truncata* and *N. menziesii*, Haase, 1992*a*, 1993; *Pseudopanax ferox*, Shepherd and Perrie, 2011). Apart from one example (*Dactylanthus taylorii*, Holzapfel et al., 2002), these studies have not focused on endangered species, rather focusing on larger scale population analysis.

At this stage, microsatellites have only been developed and published for three native New Zealand tree species, all angiosperms (*Metrosideros excelsa*, *Pseudopanax ferox*, and *Vitex lucens*), and no studies so far have applied molecular markers to the question of seed dispersal. Therefore, in this chapter I explored the feasibility of using microsatellite markers to assess dispersal of matai. Specifically I aimed to:

1. Isolate and amplify microsatellite motifs from sequenced data.
2. Test the variability of these loci across adult matai on Banks Peninsula.
3. Individually identify adult trees.
4. Genetically identify the maternal parent of dispersed seeds.

2.3 Methods

2.3.1 DNA extraction

Leaf samples were gathered from 20 trees across the Port Hills field sites and elsewhere on Banks Peninsula (Figure 2.2). All leaves were collected directly from the plant in question, to avoid mistaking leaves on the ground as coming from the incorrect tree. Due to the height of matai canopy, I collected leaves using either a slingshot or a telescopic pole with clippers attached. An additional sample was collected from a tree in Blue Duck Reserve, Kaikoura (Norton and Courtney, 2000). Leaves were immediately placed into a bag with silica gel for dessication, and stored at -20°C until used in the laboratory.

I used a modified CTAB protocol to extract genomic DNA from leaves (Weising et al., 2005). Plant material is difficult to extract purified DNA from due to thick cell walls and starches; to

improve purification, CTAB extraction involves physical destruction of plant material (e.g. with a mortar and pestle) followed by a lysis buffer (i.e. cetyl trimethylammonium bromide, CTAB) (van Pelt-Verkiul et al., 2008). The CTAB buffer was made to the following specifications: 0.1M Tris, 20mM EDTA, 1.4M NaCl, 2% CTAB.

For each sample from individual trees, I ground approximately 10 matai leaves (c. 30 mg) in a mortar and pestle with 800 µl of 2xCTAB buffer and 40 µl (0.5%) β-Mercaptoethanol. I did not allow leaves to thaw before grinding. The ground tissue and buffer was transferred to a 1.5 ml tube and incubated at 60°C for 30 min, with mixing every ten minutes. After incubation, I added 600 µl of chloroform-isoamyl alcohol (24:1), vortexed the tubes briefly, and centrifuged the tubes for 5 min at 13,000 rpm. This separates the sample into an aqueous phase containing nucleic acids, and a chloroform-isoamyl interphase containing proteins and polymers. I extracted the aqueous phase, pipetted this into a new 1.5 ml tube and repeated the chloroform-isoamyl stage to further purify the samples. After the second centrifuge (also at 13,000 rpm for 5 min) I again pipetted the aqueous phase into a new 1.5 ml tube with 4 µl of RNase A (Sigma-Aldrich), briefly spun the tube to collect contents at the bottom of the tube, then incubated at 37°C for 30 min. After incubation I added 500 µl (0.6 volume) of cold isopropanol alcohol and allowed the DNA to precipitate at -20°C for 30 min, then spun the tubes for 10 min at 13,000 rpm. At this stage the DNA forms a pellet, so I disposed of the supernatant isopropanol and washed the pellet with 300 µl of 70% ethanol followed by another 10 min spin at 13,000 rpm. I disposed of the ethanol and allowed the DNA pellet to dry for 15 min before adding 200 µl of TE Buffer and leaving the samples to resuspend overnight at 4°C. The following day I estimated DNA quantity and purity on an ND-1000 spectrophotometer (NanoDrop, Delaware, USA), which measures absorbency at set wavelengths to detect DNA, and stored the samples at -20°C.

I used the same protocol to extract DNA from matai seed coats. Seeds were collected in seed traps under female matai trees in 2009 (see Chapter 3, section 3.3.1); I used "clean" (i.e. bird dispersed) seeds with flesh already removed. I cracked seeds open using a pair of bleach-cleaned pliers, then separated the embryo from the (maternally derived) seed coat fragments and transferred the woody seed coat to a 1.5 ml tube which I then stored at -20°C. The embryo was separated from the woody seed coat by the membranous endotesta; most often the embryo was decayed and I disposed of it. The woody seed coats weighed c. 120 mg, and I used approximately half the coat (60–70 mg) for a single DNA extraction; I used liquid nitrogen to shock-freeze the seed coats prior to grinding with a mortar and pestle. After this, the protocol for DNA extraction followed that described above for leaves.

2.3.2 454 sequencing and microsatellite screening

I extracted DNA from leaf material of one matai tree from Ahuriri Valley (AV12) using the CTAB protocol described above. The sample was then subjected to 454 sequencing by the Otago Genomics facility (University of Otago, New Zealand) using a 1/16 run on a GS-FLX system (Roche, Penzberg, Germany). It is standard practice to use a single individual for 454 sequencing; of the 15 studies discussed in Guichoux et al. (2011), only one used more than one indvidual. I screened the resulting sequence library for microsatellite repeats using MSATCOMMANDER v0.8.1 (Faircloth, 2008).

An important phase of microsatellite primer development is selection of repeat motifs suitable for further development, especially so when using NGS with a huge number of repeat motifs available to choose from. Mononucleotide repeats are the most frequent microsatellites, but they are often subject to stuttering which may complicate allele scoring further down the track, particularly since allele differences may be only a single base pair (Weising et al., 2005; Selkoe and Toonen, 2006); often these repeats are eliminated from the outset (Guichoux et al., 2011). Stutter bands are a common artefact in PCR products that likely arise from slippage of the *Taq* polymerase; the bands are shorter versions of the target product for which full PCR has not been completed (Weising et al., 2005) [for an example of stutter bands, see Figure 2.4] Tri- and hexanucleotide repeats will be the most common motifs within coding regions because they do not cause a shift in the reading frame, consequently they may be under selection (Selkoe and Toonen, 2006; Gardner et al., 2011). Dinucleotides are often highly polymorphic, but they exhibit more stuttering than larger motifs (Guichoux et al., 2011; Gardner et al., 2011). Although stutter bands may create difficulties in scoring alleles, particularly if alleles in a heterozygote are close in size (and therefore the stutter from the larger allele may overlap the smaller allele), they can assist in distinguishing target products from non-specific artefacts (Guichoux et al., 2011). Gardner et al. (2011) recommend tetranucleotides as the first choice for microsatellite development due to their high polymorphism, low stutter and large allele range, but note that these are generally rare; pentanucleotides fall into this same category.

Therefore, I selected repeat motifs based on the following criteria: motifs were dinucleotide or higher, preferably with 9 or more repeats (though some tri- and tetranucleotides were selected with 7–8 repeats), and at least 20 bp of flanking region on either side of the motif to provide room for a primer. Further selection was based on the quality of the flanking region; if there were too many repeats or adenines (prone to slippage) these were deemed undesirable for primer design. Selected motifs were run through Primer3 (Rozen and Skaletsky, 2000) which designs

oligonucleotide primers; I sourced primers from Invitrogen.

2.3.3 PCR amplification

PCR amplifications of microsatellite products were run in a total volume of 15 µl, which included: 1 x *Taq* buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.33 µM of each primer, 0.6U *Taq* (Bioline) and 0.5 µl of template DNA extracted as described above (Section 2.3.1). *Taq* polymerase, derived from the thermophillic bacterium *Thermus aquaticus*, is a thermal stable enzyme allowing multiple heating and cooling cycles without denaturating the polymerase (van Pelt-Verkiul et al., 2008). Annealing temperatures (T_a, i.e. the temperature at which the primer binds to the template DNA) were calculated as 5°C below the lowest melting temperature for the primer pair (as calculated by Primer3). The standard PCR reaction cycle was: 95°C for 12 min; 10 cycles of 94°C for 15 sec, T_a°C for 30 sec, 72°C for 30 sec; followed by 30 cycles of 89°C for 15 sec, T_a°C for 30 sec, 72°C for 30 sec; then a final extension of 72°C for 10 min. All PCR reactions used a Mastercycler EP thermocycler (Eppendorf). I confirmed PCR product amplification by running samples on a 1.4% agarose gel, stained with SYBR Safe (Invitrogen), at 80V for one hour and visualising the gels on a G:Box EFS (Syngene, Maryland USA). Images were processed in GeneSys v1.0.9.0 (Syngene). PCR products should be visible on processed gel images, and by comparing the product to a standardised size ladder I was able to confirm whether the desired product had been amplified (as Primer3, used above for primer design, calculates the expected product size). Where no PCR product was visible, I lowered the T_a by 2°C and repeated the process; where multiple or smeared bands were visible, I raised the T_a by 2°C. Some loci did not amplify well under these conditions, so for these loci I increased the amount of template DNA to 1.0 µl and increased the number of cycles in the second round from 30 to 35 cycles. Higher cycles this late in the process should favour replication of the target product, since this should be in higher abundance than non-specific product.

2.3.4 Genotyping

For primer pairs that produced a PCR product, I had one primer (generally the forward primer) labelled with either 6-FAM (Sigma), VIC, NED, or PET (ABI) fluorescent dye. New combinations of unlabelled and new labelled primers were amplified and reassessed by running on an agarose gel; some pairs required further alteration to the PCR conditions. PCR products were pooled and genotyped on a capillary sequencer, ABI 3130xl Genetic Analyser (Applied Biosystems), with Genescan LIZ 500 internal size standard (Applied Biosystems) by Canterbury Sequencing and Genotyping (School of Biological Sciences, University of Canterbury). I pooled

loci with non-overlapping product sizes and only included one instance of each of the four dyes. I used GeneMarker v2.2.0 (SoftGenetics) to score loci using the number of base pairs in the PCR product as the allele size.

There are two stages in scoring loci; first, true allele calling involves reading the decimal numbers of allele peaks, second, these real-value numbers are binned into discrete units to be referred to as the allele size. This process appears to be the greatest source of discrepancy between different studies, as the binning stage is somewhat arbitrary and therefore varies between researchers and laboratories (Guichoux et al., 2011). To minimise error at this stage, I lined up all similar alleles and chose an allele bin that suited the multi-individual structure. In any case where it was unclear whether there was one allele or two, I chose to be conservative and bin them into a single allele, rather than risk inflating the total number of alleles.

There were three main cases in which allele calling was difficult: low heterozygote peak ratios, stuttering in neighbouring alleles, and split peaks; these are common problems that arise during amplification and can affect scoring (Guichoux et al., 2011). Where low heterozygote peak ratios occurred (in which mutations in the flanking regions causes low amplification in one of the two alleles present in a heterozygote) I compared this to another individual expressing the same allele, and if the allele showed the same size I scored it as a heterozygote. Split peaks occur when the *Taq* polymerase adds an extra nucleotide (normally an adenine) to PCR fragments during amplification (called adenylation) resulting in two peaks 1 bp apart (Guichoux et al., 2011). When this occurred I scored it as a single allele, as adenylation is likely to be the cause of a 1 bp difference rather than a true second allele and scoring as a single allele is more conservative [for an example of split peaks, see Figure 2.6].

I used GENALEX v6.4 (Peakall and Smouse, 2006) to calculate allele frequencies, observed (N_a) and expected (N_e) number of alleles, and observed (H_o) and expected heterozygosity (H_e) (Table 2.1). Allelic frequencies are calculated as the relative proportion of each allele in the population, such that each homozygote counts as two occurrences of a single allele. Observed heterozygosity is the proportion of individuals at a locus that are heterozygous, while expected heterozygosity is calculated as:

$$H_{\mathrm{e}} = 1 - \Sigma p_i^2$$

Where p_i is the frequency of the i^{th} allele. Expected heterozygosity assumes alleles are under Hardy-Weinberg Equilibrium (i.e. $p^2 + 2pq + q^2 = 1$); significant differences between observed and expected heterozygosity provide evidence for a deviation from Hardy-Weinberg Equilibrium. I used Arlequin v3.5.1.3 (Excoffier and Lischer, 2010) to test for deviations from Hardy-

Weinberg Equilibrium using an exact test; I used a sequential Bonferroni correction (in R v2.9.0) to account for multiple tests. While the observed number of alleles per locus (N_a) is simply the total number seen, the expected number of alleles is calculated as:

$$N_e = \frac{1}{\Sigma p_i^2}$$

Where p_i is the frequency of the i^{th} allele, and provides an estimate of the number of alleles required to provide the same heterozygosity if all allele frequencies were equal. The rationale for using N_e over N_a is that it is less sensitive to sample sizes and rare alleles, i.e. larger sample sizes are more likely to pick up rare alleles (Weising et al., 2005; Frankham et al., 2010). Additionally, I used GENALEX to search for matching multi-locus genotypes between trees.

I looked for linage disequilibrium for all polymorphic loci using Arlequin, which performs a likelihood-ratio test; the output is a p-value with no associated test statistic. I used a sequential Bonferroni correction in R (v2.9.0) on resulting p-values to account for the multiple tests. Linkage disequilibrium occurs when two or more loci interact non-randomly and are frequently inherited together. Often there is a physical association between these loci, particularly if they occur on the same chromosome, resulting in non-random assortment during recombination (Selkoe and Toonen, 2006). This may mean that neutral alleles appear to be under selection due to their association with loci that are exposed to selection (Frankham et al., 2010). Besides such physical linkage, there can also be a functional relationship between two loci or selection for the two to be passed on as a pair; both also result in linkage disequilibrium (Selkoe and Toonen, 2006). Such a functional relationship would be unusual for microsatellite loci, but it is possible that microsatellites exhibit clusters within the genome and therefore could be physically linked (Selkoe and Toonen, 2006). Linkage disequilibrium results in pseudoreplication in analyses that assume independence between loci (Selkoe and Toonen, 2006).

To assess the feasibility of using microsatellite loci to identify individuals, I calculated a Probability of Identity (P_{ID}) for each locus, and multiplicatively across all loci. P_{ID} gives the probability that two individuals randomly drawn from the same population will express an identical genotype, and is calculated as:

$$P_{ID} = \Sigma p_i^4 + \Sigma (2 p_i p_j)^2$$

Where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles (Hedrick, 2005). Multiplying the resulting probabilities across all loci then gives a multi-locus P_{ID}, although this calculation assumes all loci are independent (i.e. exhibit linkage equilibrium) and in Hardy-Weinberg Equilibrium (Taberlet and Luikart, 1999). Therefore, P_{ID} provides a measure of the power of a genetic marker to resolve between individuals drawn from a population, which will be essential in sepa-

rating potential maternal parents in studies of dispersal. In addition to calculating a multi-locus P_{ID}, I also calculated cumulative values by starting with the most polymorphic locus and adding loci sequentially.

I used 21 adult matai trees for genotyping analysis: eighteen of the adults were spread across the Port Hills study sites, two from elsewhere on Banks Peninsula (Figure 2.2 inset), and a single adult from Blue Duck Reserve, Kaikoura. For all 21 adults, I ran PCR for all fluorescently labelled loci, and genotyped those that amplified. Only the 20 Banks Peninsula individuals were used for population statistics (e.g. Hardy-Weinberg equilibrium). To investigate the feasibility of using the method of Godoy and Jordano (2001) for genotyping matai seeds, I collected seeds caught under female matai trees (see Chapter 3, Section 3.3.1) and attempted to genotype these seeds at the same loci the adults were assessed at.

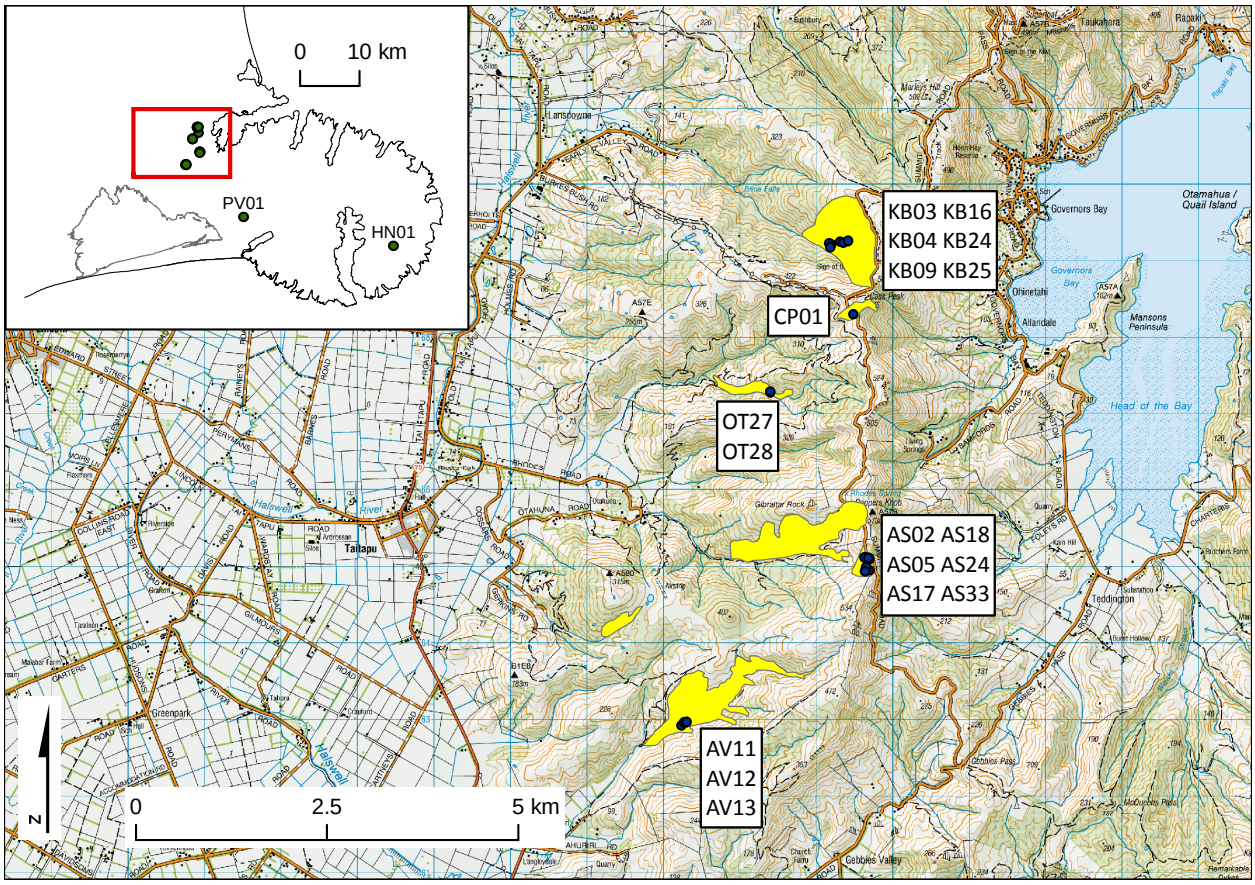


Figure 2.2: Location of 20 Banks Peninsula adult matai trees used in genotyping study. PV01 and HN01 refer to Prices Valley and Hinewai Reserve. For details of the Port Hill sites, see Figure 1.1, pg. 20.

2.4 Results

2.4.1 454 sequencing and microsatellite screening

For a small sample (n = 27) of leaf extracts, I calculated summary statistics of DNA yield and quality: overall, yield was low ($\bar{x}$ = 54.8 ng/µl $\pm$ 3.6 SE). I measured purity by assessing OD260:280 ratios, these were also low ($\bar{x}$ = 1.42 $\pm$ 0.05 SE). Pure DNA extractions should show an OD260:280 ratio between 1.8–2.0, lower values indicate protein or phenol contamination while higher ratios suggest the presence of RNA (Weising et al., 2005).

From the 454 sequencing, 21,692 sequences were obtained; screening with MSATCOMMANDER v0.8.1 (Faircloth, 2008) found 564 repeat motifs (across 504 sequences). These repeats consisted of 147 mononucleotides, 187 dinucleotides, 201 trinucleotides, 24 tetranucleodites and 5 hexanucleotide loci; there were no pentanucleotide loci.

The average fragment size was 179 bp, shorter than that found across 13 plant species by Gardner et al. (2011) ($\bar{x}$ = 336 bp). This may indicate degradation of my DNA samples during extraction, resulting in shearing. It is estimated than when the average fragment size is around 200 bp, some two-thirds of microsatellites will be too close to either end of the fragment to allow primer design in the flanking regions (Guichoux et al., 2011). In my data, this was reflected in the high proportion of repeat motifs that were unsuitable for primer development.

I conducted two rounds of primer development. In the first round, I designed primer pairs for 21 microsatellite loci and worked through the optimisation process (finding annealing temperatures that give a clear PCR product). Of these, eight amplified and I labelled these loci with fluorescent dyes for genotyping. Based on these results, I designed primers for nine new loci and redeveloped primers (i.e. using a different section of the flanking region) for nine loci from the first set, for a total of 18 new primer pairs; from these, a further eight loci amplified and I also labelled these, giving a total of 16 primer pairs for genotyping.

2.4.2 Microsatellite primer development

Of the 16 fluorescently labelled loci, ten amplified consistently enough to enable genotyping. Of these, three loci were monomorphic across the 20 Banks Peninsula individuals, while another two were heterozygous for one individual each, even with the Kaikoura individual included (Tables 2.1 & 2.2). Polymorphism is defined as having more than one allele, with the most common allele exhibiting no more than 95% (or sometimes 99%) frequency (Frankham et al., 2010); due to the small sample size of my study, I have chosen to treat these two loci as functionally monomor-

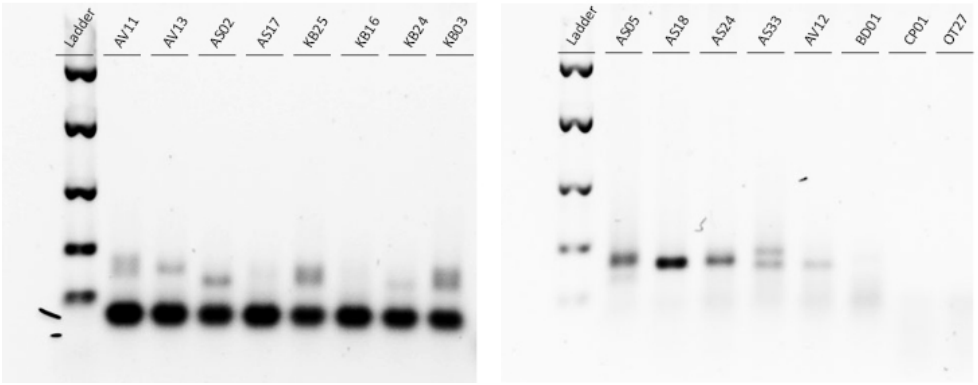
Table 2.1: Microsatellite loci isolated from *Prumnopitys taxifolia*. T_a annealing temperature, N_a number of alleles, H_O observed heterozygosity, H_E heterozygosity expected under Hardy-Weinberg Equilibrium.

Locus	Repeat motif	Dye	Primer sequence (5'-3')	T_a°C	N_a	Size range (bp)	H_O	H_E
Ptx10	[AC]23	VIC	F: AAAAGTAACACAAAGAGATAGTGCT	54	12	138–178	0.600	0.864
			R: GGCACCCCTTTCGTATGTTT					
Ptx19	[AT]9	VIC	F: GTTCCTAAGAGGACGAATGCTT	56	11	172–234	0.667	0.876
			R: CATGATCGAGTCACCTATTGGA					
Ptx8	[GT]12	6-FAM	F: GACACATGAGTCATCGCAAAA	54	7	120–175	0.400	0.539
			R: GGTCGATAGTGTCTTATGGTGACA					
Ptx20a	[AC]12	NED	F: ATGCGCATGTGCACGC	54	5	115–123	0.850	0.647
			R: CATAAACCACAATGGGCTCA					
Ptx14	[AAT]10	NED	F: TGATCAATATTCTTGTTAATTCATGT	54	4	81–96	0.600	0.525
			R: TTGACCGTTTTCTGTCACCA					
Ptx26	[ATC]16	6-FAM	F: TCCTACAATGACACCCTTTA	47	2	191–202	0.050	0.049
			R: TGTGTTGATGATTATTAATATTAT					
Ptx28	[GT]8	PET	F: TGAAATATTTTAATTCATATGTG	48	2	181–183	0.050	0.049
			R: GAGCTTGAATACTCCAAAAG					
Ptx4	[AT]12	PET	F: AAAATAAGTGAATTAGAAGACAACTGC	54	1	101	0	0
			R: TGAGGGAAAACATGAACACAA					
Ptx9	[AT]9	NED	F: ATGCATCATTCATAAAGGTTCATC	56	1	215	0	0
			R: TTGGTTGCAACATGTTCGTT					
Ptx21	[GT]16	6-FAM	F: CTGCATGTAATTGGGTCT	51	1	114	0	0
			R: CTACTAATGGAGAACTTACAACA					

phic since their most common allele had 98% frequency (Table 2.3). This then gives a percentage polymorphism across the ten loci of 50%. Mean number of alleles (N_a) per locus was 4.6 ($\pm$ 1.3 SE), while the mean effective number of alleles (N_e) was 2.7 ($\pm$ 0.8 SE). Of the five polymorphic loci, two (Ptx10, Ptx19) showed significant deviations from Hardy-Weinberg Equilibrium (Table 2.4). Both exhibited a heterozygote deficiency; that is, expected heterozygosity was significantly higher than that observed. One pair of loci (Ptx10, Ptx14) showed signs of linkage disequilibrium ($p = 0.009$), however after I corrected for multiple testing this pair was no longer significant ($p = 0.09$). There were no significant results between any other pairs of polymorphic loci.

Table 2.2: Multi-locus genotypes for 21 matai across 10 microsatellite loci. Names for each tree are coded by the site of origin: Ahuriri Summit (AS), Ahuriri Valley (AV), Cass Peak (CP), Hinewai Reserve (HN), Kennedys Bush (KB), Otahuna Reserve (OT), Prices Valley (PV), Blue Duck Reserve, Kaikoura (BD). Rare alleles in loci Ptx26 and Ptx28 are highlighed in bold. Missing data (-) is due to failure during PCR amplification.

Individual	Ptx4	Ptx8	Ptx9	Ptx10	Ptx14	Ptx19	Ptx20a	Ptx21	Ptx26	Ptx28
Banks Peninsula										
AS02	101/101	120/120	215/215	140/140	81/93	172/174	119/121	114/114	191/191	181/181
AS05	101/101	130/130	215/215	146/150	81/93	186/199	119/121	114/114	191/191	181/181
AS17	101/101	120/120	215/215	140/160	84/93	176/214	119/123	114/114	191/191	181/181
AS18	101/101	120/123	215/215	160/160	93/96	181/183	119/119	114/114	191/191	181/181
AS24	101/101	120/120	215/215	150/154	81/93	183/183	119/123	114/114	191/191	181/**183**
AS33	101/101	120/120	215/215	146/160	93/96	174/214	119/119	114/114	191/191	181/181
AV11	101/101	120/120	215/215	158/178	81/93	172/172	117/119	114/114	191/191	181/181
AV12	101/101	123/135	215/215	150/156	96/96	174/174	117/119	114/114	191/191	181/181
AV13	101/101	120/123	215/215	140/172	93/93	186/214	115/117	114/114	191/191	181/181
CP01	101/101	120/120	215/215	142/154	93/96	-/-	117/119	114/114	191/191	181/181
HN01	101/101	120/175	215/215	146/160	93/93	-/-	119/121	114/114	191/191	181/181
KB03	101/101	120/130	215/215	138/154	93/96	186/225	119/121	114/114	191/191	181/181
KB04	101/101	123/149	215/215	154/154	93/93	172/201	119/119	114/114	191/**202**	181/181
KB09	101/101	120/123	215/215	149/154	84/93	-/-	119/123	114/114	191/191	181/181
KB16	101/101	120/120	215/215	154/154	93/93	-/-	117/119	114/114	191/191	181/181
KB24	101/101	120/120	215/215	140/140	93/93	176/176	117/119	114/114	191/191	181/181
KB25	101/101	120/120	215/215	140/156	93/96	174/236	117/119	114/114	191/191	181/181
OT27	101/101	120/120	215/215	158/158	93/93	172/172	119/123	114/114	191/191	181/181
OT28	101/101	120/127	215/215	140/140	93/93	-/-	117/119	114/114	191/191	181/181
PV01	101/101	123/123	215/215	156/156	93/96	186/199	119/123	114/114	191/191	181/181
Kaikoura										
BD01	101/101	124/130	215/215	151/157	93/93	191/191	119/121	114/114	191/191	181/181



(a) Ptx10 **(b)** Ptx19

Figure 2.3: Ptx10 and Ptx19 exhibiting double bands, primer dimer and individual amplification failure.

2.4.3 Issues with amplification

Although I tested 30 primer pairs, only ten successfully amplified. Of the nine loci that I redesigned primers for, only one (Ptx20a - the 'a' denotes a second primer) amplified successfully on the second trial. Additionally, of the 16 loci that I had fluorescently labelled, six did not amplify sufficiently to allow genotyping. Such issues with amplification are likely due to mutations in the flanking regions on one or both sides of the microsatellite (van Pelt-Verkiul et al., 2008). With more time, further optimisation could improve amplification of these loci; however, it appeared that there was enough polymorphism in the loci I had working to carry on with the study.

Of the five polymorphic loci, two (Ptx10, Ptx19) amplified inconsistently. Figure 2.3 is an example of agarose electrophoresis results showing certain individuals failing in a run of otherwise successful PCR amplification (e.g. AS17 and KB16 in Ptx10). These inconsistent results likely reflect poorly designed primers or flanking sequence mutations, both which could be addressed with further optimisation. Amplification in Ptx10 may also have been affected by primer dimer (see below). Such amplification failure greatly increases the laboratory time required for genotyping and is one of the reasons primer optimisation took much longer than expected; Ptx10 and Ptx19 have the highest number of alleles per locus (Table 2.1) so until these alleles began amplifying more consistently I did not have enough information to determine their polymorphism. Additionally, there are issues with replicability of results if these amplification issues are not addressed.

Along with inconsistent amplification, Ptx19 also showed "short allele dominance" due to the large base pair distance between some alleles. Short allele dominance occurs when the two alleles present in a heterozygote do not amplify at similar levels; the PCR process is more efficient

for shorter alleles, so when there is a pronounced difference in allele sizes, the larger allele will amplify less (Selkoe and Toonen, 2006). Evidence of short allele dominance in Ptx19 is presented in Figure 2.4; note the two "large" alleles (201, 215) have amplified no more than one-third the intensity of the short alleles (172, 175, 188).

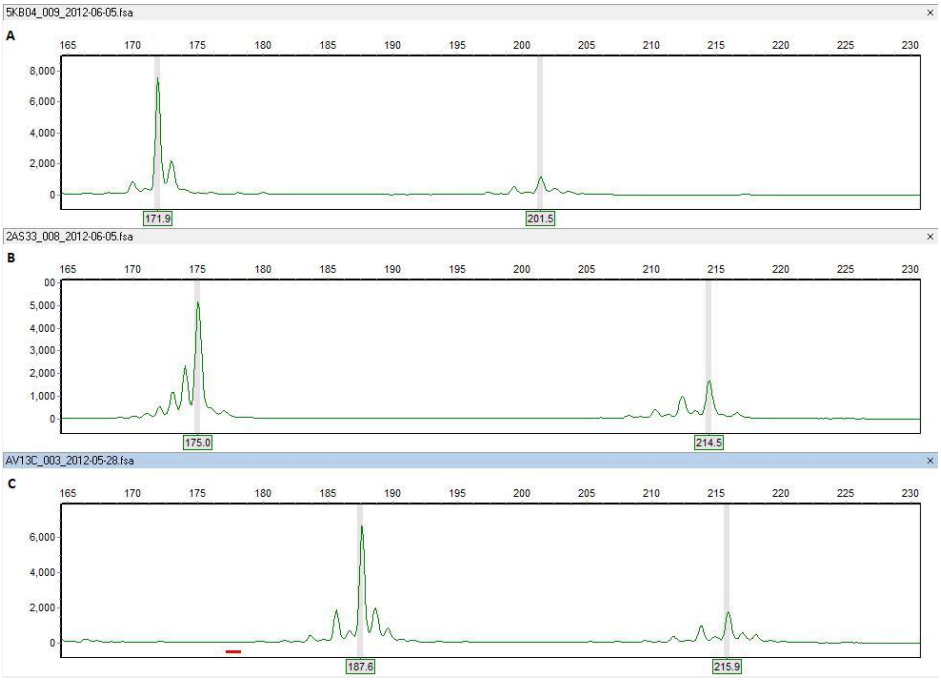


Figure 2.4: Evidence of short allele dominance at a microsatellite locus (Ptx19) amplified in three individuals (A: KB04, B: AS33, C: AV13). Allele size (bp) is shown on the x-axis, with fluorescent intensity (Relative Fluorescent Units - RFU) on the y-axis. Note the stuttering evident in the 175 bp allele of AS33.

If primer pairs have complementary regions, "primer dimer" may form; this is primer-primer hybridisation and is generally 50–100 bp in length. As the PCR process is more efficient for shorter products, the amplification of this primer dimer may occur at the expense of the desired product (van Pelt-Verkiul et al., 2008). In some loci, this primer dimer was so prevalent there was no obvious amplification of the target product; in the case of one locus, the desired product was small (c. 80 bp) and indistinguishable from the primer dimer (c. 70 bp) when genotyped. An example of primer dimer in Ptx10 is shown in Figure 2.3; for this locus, raising the annealing temperature in order to increase specificity resulted in a complete loss of target product, so I could not reduce the amplification of primer dimer. Such non-target amplification probably reduces the expression of the target product (van Pelt-Verkiul et al., 2008), and may be part of the reason Ptx10 amplified inconsistently and was sometimes too low to accurately score.

A final phenomenon visible in Figure 2.3 is that in some cases heterozygotes can be identified from the electrophoresis results. For those loci with marked differences in allele sizes, heterozy-

gotes are sometimes evident after running PCR products on an agarose gel. For example, alleles at both Ptx10 and Ptx19 exhibit a wide range of product sizes (Table 2.1) and individuals with one allele from either end of the size range are visible in gel results (Figure 2.3). For Ptx10, three heterozygotes are identifiable in the gel results (AV11, KB25, KB03) and AS33 is heterozygous for Ptx19 (see Table 2.2 for genotypes).

2.4.4 Identification of individuals

Of the 20 genotyped Banks Peninsula trees, none shared a multi-locus genotype (Table 2.2), enabling each tree to be individually identified. The individual from Kaikoura (BD01) exhibited a single allele at locus Ptx19 that was not present in any of the Banks Peninsula individuals (Table 2.2). However, given the high allelic diversity at this locus and the lower sample size (due to amplification failure), it is possible that with a larger sample this additional allele would show up in the Banks Peninsula population as well, rather than being a "private" allele to Kaikoura. While two loci were functionally monomorphic in my samples (Ptx26, Ptx28; Tables 2.3 & 2.2), these rare alleles may show up more frequently in a larger sample size, and could be useful for distinguishing otherwise identical individuals in a large population.

Probability of Identity analysis indicates that the five polymorphic loci will be sufficient to identify individual adult matai. Because Ptx19 showed amplification problems, I also calculated cumulative P_{ID} without Ptx19; these values, although a magnitude larger, still suggest the ability to identify individuals (Table 2.5).

2.4.5 Seed coat amplifications

Seed coat extractions regularly exhibited brown discolouration and viscous consistency; these extractions were unusable for PCR amplification. They showed abnormally high DNA quantities (up to 350 ng/µl cf. $\bar{x}$ = 55 from leaf extracts) and occasionally failed to read on the spectrophotometer entirely. Extractions that did not show this discolouration exhibited far lower yields ($\bar{x}$ = 9 ng/µl). Such extreme differences indicates an overestimation of seed coat yield, most likely due to some contamination in the DNA extractions - in this case, likely secondary compounds. Of 32 seed coat extractions I conducted in June 2012, only eight (25%) amplified at tested loci. These eight extractions had lower DNA concentrations ($\bar{x}$ = 9.24 $\pm$ 1.45 SE ng/µl) than non-amplifying seeds, and the OD260:280 ratios were on the same scale as leaf extractions ($\bar{x}$ = 1.46 $\pm$ 0.12 ng/µl).

Low yields from seed coat extractions are not unusual: Godoy & Jordano (2001) obtained yields of 1–5 ng/µl from endocarp extracts, concentrations too low to visualise on a gel but they were still able to successfully amplify microsatellites from these concentrations.

Of the small set of seed coats I genotyped, amplification was generally much lower than that seed from leaf extracts. This made scoring loci for seed extracts much more difficult. For example, in Figure 2.5 I have presented the electropherogram results from one tree (AS17) and two seeds collected from seed traps under the canopy, therefore treating AS17 as the putative parent. AS17 is heterozygous at Ptx20a for alleles 119 and 123; both seeds express the allele at 119, but it is unclear whether they have a second allele at 123. To be consistent in loci scoring, I would have to categorise both seeds as homozygotes. If their genotypes were actually 119/123, there are only five matching trees across the 20 genotyped adults (Table 2.2), and only two of these are within the same field site (AS17, AS24); however, if we can only read the 119 allele, 19 of the 20 adults had the same allele, vastly reducing exclusion power of maternal assignment.

A second example is presented in Figure 2.6 with a different tree (AS24) and locus (Ptx14). AS24 is heterozygous for alleles 81 and 93; the two seeds presented (S2942, S3155) are putatively the offspring of this tree, but both are amplifying only one allele clearly. Again, this amplification precludes confident assignment of seeds to their presumed parent.

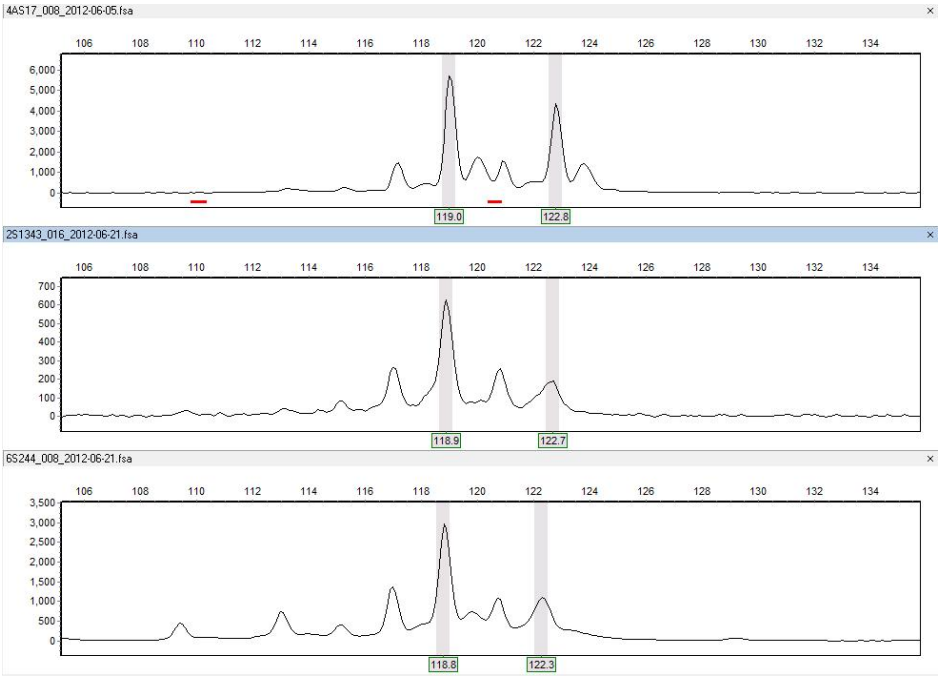


Figure 2.5: Electropherogram of one tree (AS17 - top) and two seeds (S1343, S244) at a microsatellite locus (Ptx20a). AS17 is heterozygous at alleles 119 and 123; however, low amplification for both seeds makes it unclear whether they are homozygous (119) or also heterozygous. Allele size (bp) is shown on the x-axis with fluorescent intensity (RFU) on the y-axis: note the varying y-axes scales.

Unfortunately, due to seismic disruptions in our Christchurch based laboratory in 2010 and 2011, it took much longer than expected to optimise primers and gather enough information

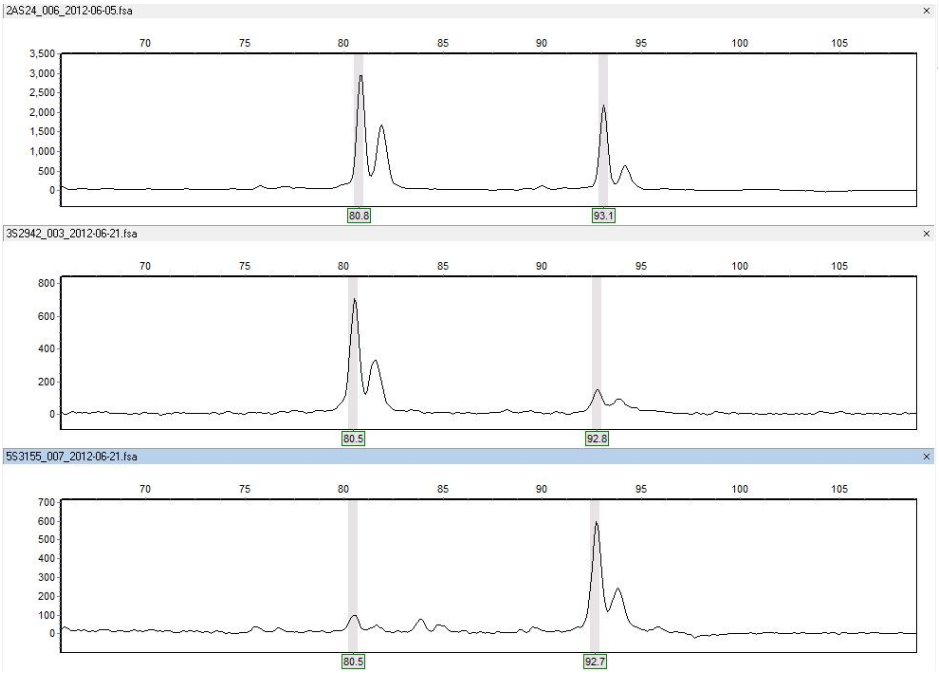


Figure 2.6: Electropherogram of one tree (AS24 - top) and two seeds (S2942, S3155) at a microsatellite locus (Ptx14). AS24 is heterozygous at alleles 81 and 93; however, low amplification for both seeds makes it unclear whether they are homozygous at 81 and 93 respectively or also heterozygous. Allele size (bp) is shown on the x-axis with fluorescent intensity (RFU) on the y-axis: note the varying y-axes scales. Both alleles exhibit split peaks caused by adenylation.

to conclude that polymorphism rates across my loci were high enough for individual identification. Once this was determined, I had very little time left to carry on with DNA extractions, PCR amplification and genotyping. This prevented me from further refining the extraction method for seed coats, which may have allowed better yields and amplification. This would then have permitted assignment of maternal identity for a larger sample of seeds.

Table 2.3: Allelic frequencies at ten amplified microsatellite loci from *Prumnopitys taxifolia.* Loci marked * are weakly polymorphic, I have treated them as monomorphic in calculations of polymorphism. Sample sizes are 20 for all loci except Ptx19 (n = 15).

Locus	Allele	Frequency	Locus	Allele	Frequency
Ptx4	101	1.00	Ptx19	172	0.20
Ptx8	120	0.65		174	0.17
	123	0.18		176	0.10
	127	0.03		181	0.03
	130	0.08		183	0.10
	135	0.03		186	0.13
	149	0.03		199	0.07
	175	0.03		201	0.03
Ptx9	215	1.00		214	0.10
Ptx10	138	0.03		225	0.03
	140	0.23		234	0.03
	142	0.03	Ptx20a	115	0.03
	146	0.08		117	0.20
	149	0.03		119	0.55
	150	0.08		121	0.10
	154	0.20		123	0.13
	156	0.10	Ptx21	114	1.00
	158	0.08	Ptx26*	191	0.98
	160	0.13		202	0.02
	172	0.03	Ptx28*	181	0.98
	178	0.03		183	0.02
Ptx14	81	0.10			
	84	0.05			
	93	0.65			
	96	0.20			

Table 2.4: Test for deviation from Hardy-Weinberg equilibrium, p-values from Arlequin's exact test before and after sequential bonferroni correction for microsatellite loci isolated from *Prumnopitys taxifolia*. NB: H_E values differ from Table 2.1 due to differences in calculation. Values in bold indicate significance at α = 0.05.

Locus	H_O	H_E	p-value	Corrected p-value
Ptx8	0.400	0.553	0.110	0.330
Ptx10	0.600	0.886	0.003	**0.015**
Ptx14	0.600	0.538	0.855	0.855
Ptx19	0.667	0.906	0.008	**0.032**
Ptx20a	0.850	0.647	0.146	0.330

Table 2.5: Probability of Identity (P_{ID}) for polymorphic loci in *Prumnopitys taxifolia*; loci are ordered from highest polymorphism to lowest. P_{ID} is expressed as the estimated proportion of individuals sharing a genotype at each locus. Observed P_{ID} is calculated as the proportion of all possible pairs of individuals that shared a genotype at each locus. Cumulative P_{ID} is calculated by adding loci sequentially, highest polymorphism to lowest, and recalculating P_{ID} at each step; I calcuated this twice, once with and once without Ptx19. The final cumulative value (in bold) represents the multiplicative value across all loci.

Locus	n	P_{ID}	Observed P_{ID}	Cumulative P_{ID}	Cumulative P_{ID} (no Ptx19)
Ptx10	20	0.03	0.03	0.03	0.03
Ptx19	15	0.03	0.02	0.0009	-
Ptx8	20	0.25	0.25	0.0002	0.008
Ptx20a	20	0.18	0.21	0.00004	0.001
Ptx14	20	0.27	0.23	**0.00001**	**0.0004**

2.5 Discussion

2.5.1 Amplification of loci

Through the process of primer optimisation, many suboptimal primers are discarded along the way. Of the 564 repeats obtained through 454 sequencing, I found 30 that were suitable for primer development (5%); of these 30, only ten amplified well enough to enable genotyping. A study on weta (*Hemideina* sp.,) conducted in the same laboratory began with 31 loci, of which 14 amplified and 12 were polymorphic (Hale et al., 2010). Such high fallout of loci is an unfortunate consequence of microsatellite development, particularly with high-throughput sequencing which may frequently cut repeat motifs either partway through the repeat or without sufficient flanking regions for primer development.

Several loci showed issues in amplification, whether through complete failure, inconsistent amplification or messy scoring. Because of this it took much longer than anticipated to optimise primers to a point where I was able to genotype sufficiently large numbers of adults and determine polymorphism rates.

Short allele dominance, as evidenced in Ptx19, may result in erroneously classing heterozygotes as homozygotes, and where this is thought to be an issue homozygotes should be re-amplified with either higher concentration in the sequencer or with longer extension times during PCR (Selkoe and Toonen, 2006; Wattier et al., 1998). If there is such mistaken identity of heterozygotes, the result will be a heterozygote deficiency (Wattier et al., 1998). In the case of Ptx19, four of the 15 genotyped individuals were scored as homozygotes (Table 2.2); if this locus were to be used in further studies, it would be prudent to further assess the possibility of misidentification. In fact, Ptx19 does show a deviation between observed and expected heterozygosity (Table 2.4) which may be an artefact of short allele dominance. Due to the inconsistent amplification of this primer, along with potential issues with short allele dominance and therefore heterozygote deficiency, I would not recommend this locus be used for further study unless these issues can be cleared up through further primer optimisation. Additionally, Ptx10 shows a heterozygote deficiency (Table 2.4), but with the allelic size range spanning 40 bp, compared to a range of 60 bp for Ptx19, it is not evident whether this is enough to cause short allele dominance.

2.5.2 Seed coat

Although I have successfully isolated and amplified microsatellite loci in matai, further optimisation is required before these loci can be applied to dispersal related questions, and due to time

restrictions this falls out of the scope of my thesis. Contamination in seed coat DNA extractions is a huge barrier to successful and consistent PCR amplifications, and until this protocol has been further optimised it is unfeasible to continue with genotyping seed coats. Particularly with the low seed fall of matai across multiple years (see Chapter 3), seed samples are too precious to expend on a protocol with such a low success rate. However, my research paves the way for further studies to pick up where I have left off and expand upon my data.

Plants produce a range of secondary compounds (e.g. phenols, terpenes and alkaloids) which can influence DNA extractions. Phenols and polyphenols are particularly problematic for DNA extraction as they readily oxidise and form quinonic compounds that, along with causing a brown discolouration of DNA extractions, can damage DNA and proteins and may affect enzymatic function (Weising et al., 2005; Maltas et al., 2011). Additionally, polysaccharides tend to co-isolate with DNA, causing a highly viscous solution, and may inhibit *Taq* polymerase (Weising et al., 2005).

Contamination by these secondary compounds is the most likely cause of difficulties in extracting DNA from seed coats. Seed coat extracts were often brown coloured (phenol contamination) and highly viscous (polysaccharide contamination); PCR amplification of these discoloured extracts were always unsuccessful. In the minority of samples that did not show this contamination, PCR amplification occurred albeit inconsistently and with low amplification.

There are several options to improve DNA extractions when secondary compounds are an issue. The addition of soluble polyvinylpyrrolidone (PVP) to CTAB buffer can aid removal of polyphenols; the PVP acts as a polyphenol adsorbent and accumulates at the interphase during chloroform extraction (Weising et al., 2005; Maltas et al., 2011). Higher concentrations of antioxidants (in my protocol, β-Mercaptoethanol) may inhibit oxidation of polyphenols, while increasing the ratio of buffer to tissue may reduce the effects of polyphenols through dilution (Weising et al., 2005). Finally, elevated concentrations of CTAB buffer (e.g. increase from 2% to 4%) or increasing the level of NaCl (e.g. from 1.4M to 2M) may assist removal of polysaccharides (Weising et al., 2005; Sá et al., 2011). With further time, experimenting with these options will likely improve the quality of seed coat extractions and enable cleaner amplification of microsatellites.

2.5.3 Future directions

I have found polymorphic loci in matai that are sufficient to identify individual trees. Although I was unable to fully develop the system, preliminary results suggest these loci will be useful for maternal analyses. The next step is to optimise DNA extractions from seed coats so that

confident genotypes can be gained from seeds. Then a full verification of the system requires confirming that accurate maternal assignment can be made; the best way to do this would be to collect seeds directly from the tree canopy, or genotyping seeds that have fallen to the ground with the flesh still attached. To my knowledge, this is only the third New Zealand native tree to have microsatellites developed, and the first New Zealand study to explore prospects for using maternal tissues of seeds to determine maternal parents. These techniques have great potential for revealing detail of seed dispersal distances under complex field conditions, including across fragmented landscapes and with a range of animal dispersal agents. I discuss further applications for these microsatellite markers in Chapter 6.

Chapter 3

Movement of matai seed within forest fragments

Ahuriri Summit podocarps: totara (left), kahikatea (right), matai (background right).

3.1 Abstract

Fragmentation may lead to reduced seed dispersal of large-seeded plants if fragments contain lower abundances of large-bodied frugivores. I quantified dispersal and seed predation of matai on the Port Hills using seed traps and ground plots in 2009 and 2010. Over two years I collected seeds falling directly beneath female matai trees and categorised seeds as dispersed, undispersed or predated. Mean seed fall beneath individual females ranged from 520–1543 seeds per m^2. I used the percent of "clean" (i.e. dispersed) seeds as a proxy for dispersal service, though this cannot account for seeds moved away from the tree. Mean percent of clean seed beneath female was 47.8–54.8% in 2009, and 18.7–23.4% in 2010. Insect predation, presumably by the larvae of *Heterocrossa iophaea*, formed the largest barrier to late fruit development but varied between years: in 2009 mean insect predation was 0–3% depending on site, but this increased to 13.5–19% in 2010. Pre-dispersal rat predation affected a small percent of seeds (mean 0.3–1.5%); seeds collected from the ground exhibited slightly higher rat predation (4.2–4.9%), this was still lower than insect predation (7.1–9.1%). Seed traps spread across Port Hills sites caught a total of 84 seeds in 2009, 98% of these seeds were caught within 55 m of the nearest female matai with a maximum distance of 130 m. An additional 76 seeds were caught along 25 m transects with a trap every 5 m from the trunk of a female matai: 83% of the seeds were caught in traps 5 m from a tree (i.e. under the canopy); from 15–25 m, all seeds had been passed through a bird.

3.2 Introduction

Seed dispersal is intricately linked to and reliant on the production of fruit. Through flower production, pollination, fruit growth and pre-dispersal predation, any disruption to the process can reduce the amount of fruit available for dispersal (Figure 3.1). Each step can be seen as a series of filters, affecting eventual fruit set. A species will have evolved to cope with natural levels of fruit failure or predation and still maintain sufficient germination to self-replace. Introduction of new species, however, may have altered the dynamic and could potentially disrupt mutualism processes.

Prior to human arrival, New Zealand plant species would have been exposed to avian (e.g. Psitticidae; Clout and Hay, 1989) and insect (Sullivan et al., 1995) seed predators. Introduced mammals could act as either seed predators or dispersers, but rodents appear to be primarily seed predators. Ship rats (*Rattus rattus*) are the most common rodent species in New Zealand forests and are able to predate seeds in the canopy (pre-dispersal) or after fruits have fallen to

the ground (post-dispersal). Pre-dispersal predation reduces the number of fruits available for native frugivores, while both stages of predation reduce the overall amount of seed available for germination. Whether this affects adult plant density depends on whether a given species is seed limited (i.e. more available seed results in higher recruitment) (Münzbergová and Herben, 2005).

During the fruit development stage, fruits may be predated upon before they are able to ripen, they may abort due to environmental conditions (e.g. drought), or be physically knocked from the canopy (e.g. by strong winds). Such fruits do not ripen and thus are lost to further stages. Once ripened, fruit may still be predated while in the canopy, or knocked from the tree before being removed. Such ripened fruit falling to the ground may be available to seed predators or dispersers.

Matai is known to have at least one insect seed predator, *Heterocrossa iophaea* (Lepidoptera: Carposinidae), a moth whose larvae feeds on seeds (Sullivan et al., 1995), and this was presumed to be the seed predator in this instance though I did not rear adults to confirm. *Heterocrossa iophaea* feeds on matai seeds by eating a hole in the thick seed coat, entering the seed and feeding on the contents. Predated seeds can be identified by the presence of at least one small hole in the seed coat through which the moth larvae entered. It appears that *H. iophaea* can chew through the seed coat early in the seed's development, but once the flesh starts ripening to black and the seed coat thickens the insect no longer enters the seed, instead feeding on the flesh (Sullivan et al., 1995).

As early as 1912, seed predation on matai was remarked upon, with most ovules examined by Gibbs (1912) "attacked by some insect grub, aborted and abnormally swollen" (cited in Salter 2004, page 37). "Most to many" of young ovules collected were found to be occupied by larvae, of an undescribed species of gall midge in the family Cecidomyiidae (Salter, 2004). It appears eggs are laid in spring inside the young ovules while their micropyles are open for pollination, the larvae pupate inside with adults emerging the following spring (Salter, 2004).

By examining several stages of fruit development I sought to determine the major filters in matai development. The long period between pollination and fruit ripening in matai (18 months) and canopy height prevented inclusion of early fruit development in this project. Instead I focused on the period after fruit began to fall from the tree. Specifically I asked the following questions:

1. How much fruit falling from trees has failed or been preyed upon, and how does this affect fruit availability for dispersers?

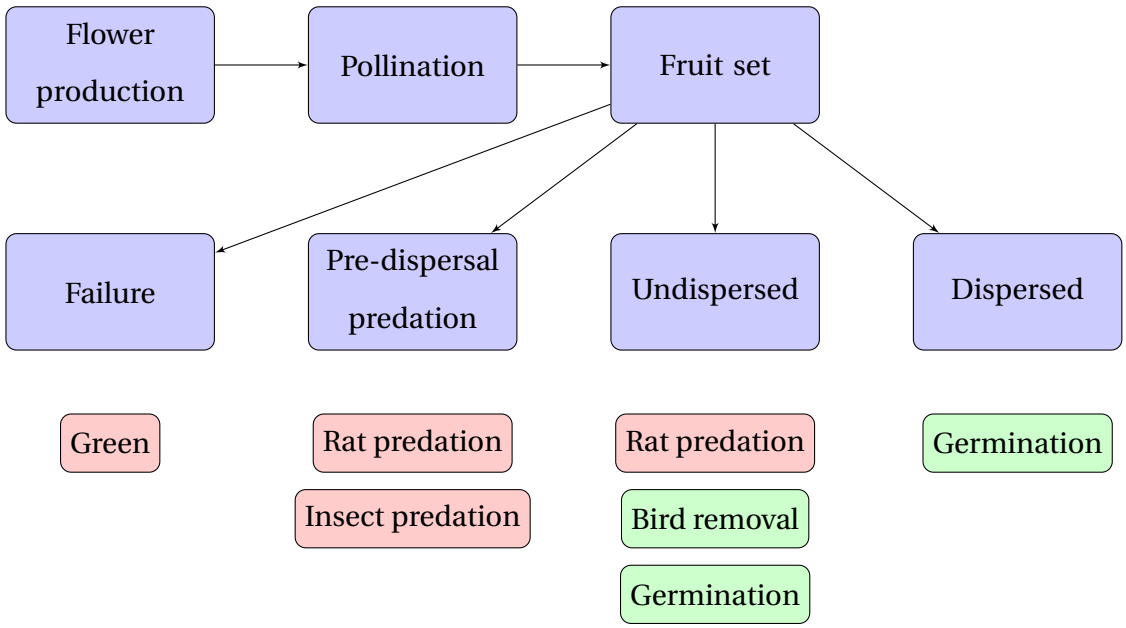


Figure 3.1: Process of fruit/seed fate. Red outcomes indicate cases where germination is prevented, while green outcomes have the potential to germinate, whether dispersed or not.

2. Is there evidence for pre-dispersal predation by examining seeds on the ground?
3. What is the fate of fruits that fall off the tree intact?
4. How much of the seeds falling under trees have been removed by dispersers?

In addition to how seeds are treated by predators, once a seed is removed by a legitimate disperser there are considerations with regard to where seeds are deposited. For example, if post-dispersal seed predators show a functional response to seed density, movement of seeds away from the parent (thus reducing spatial clumping) may reduce risk of predation (Janzen, 1970; Connell, 1971). Even without knowing what species removed a seed, we can assess spatial deposition of seeds and their fate at those sites (e.g. predated, germinated). Thus, I also aimed to answer the questions:

5. How far are seeds moved by avian dispersers?
6. After dispersal, how does habitat and distance from female trees affect germination and predation?

3.3 Methods

3.3.1 Seed traps

Standard plastic household buckets were used as seed traps in this study; buckets measured 24 cm high with a diameter of 26 cm, giving an area of 0.053 m^2. A trial of the buckets was done in Ahuriri Summit in January 2009. Ten buckets had holes drilled in the centre and were pegged into the ground using a length of 8 gauge wire (diameter = 4 mm). Buckets were set up in pairs, approximately one metre from each other. Each bucket had a piece of apple and a piece of cheese put both inside and immediately outside the bucket. One bucket in each pair had Liquid Shotgun (Southern Woods Nursery, NZ) painted around the rim. Liquid Shotgun is an egg-based repellent for possums, rabbits and hares. The buckets were checked for food removal or disturbance by mammals after four and ten nights. I found no evidence that animals were able to climb into the buckets and remove food items without tipping the bucket over. None of the buckets had contents removed from within, despite all of the buckets having at least the apple removed from outside after ten nights. There was no difference between buckets with and without Liquid Shotgun, so it was not used for any further seed trapping.

Based on the above trial, a trap design was settled on. Buckets had a single hole drilled in the centre of the bottom with another 3–4 holes drilled around the edge of the bucket bottom for water drainage, and were pegged to the ground with a length of 8 gauge wire. To facilitate seed collection, fine-mesh nylon curtain material was used to make bags to sit within the buckets. Bags measured approximately 30 cm deep by 85 cm and were fastened around the bucket rim using a rubber band (Figure 3.2a).

Seed traps were set up under fruiting female matai trees in two years, 2009 and 2010. Two sites were used, Ahuriri Summit and Kennedys Bush, with five female trees from each site used for both years. Three traps were placed under each female, for a total trap area of 0.16 m^2 per tree (Figure 3.2b). In 2009, traps were set up in March, as fruiting was beginning. They were checked monthly until July 2009, then the mesh bags were removed but the buckets were left set up for the remainder of the year. In 2010, any contents in the buckets was collected in January and the mesh bags reinstalled, meaning an extra two months of data was collected compared to 2009. The buckets were again checked monthly until July. Seeds collected from traps were kept refrigerated for several days until they could be counted. All seeds were examined for signs of predation (insect, rat), and classed as clean (dispersed, flesh removed) or undispersed (flesh still attached). While the outer layers of the epimatium in matai seeds (see Chapter 2) ripen to a

(a) Seed trap **(b)** Three traps placed under a female matai tree

Figure 3.2: Examples of seed traps used in the study

purplish-black colour, the inner layers remain green; these inner layers adhere to the seed coat and have a mucilaginous consistency which is difficult to remove (Salter, 2004). I have observed that recently bird-dispersed seeds, though having had the "skin" removed, still have this inner epimatium attached, giving them a slimy feel and an easily identifiable trait (in fact, they can be quite hard to handle without dropping). Clean seeds (i.e. dispersed) were placed into a zip-lock bag with silica gel and stored at -20°C for use in genetic analysis of seed dispersal (Chapter 2).

In Ahuriri Summit, grid traps were set up next to previously located ground plots in February 2009 (Section 3.3.2) for a total of 52 traps spread throughout the site. Additionally, in March 2009 traps were set up in a stratified manner throughout all field sites on the Port Hills (Appendix A) for a total of 356 traps. In February 2010, based on the trap results from the previous year, trap effort was concentrated in fenced sites (Ahuriri Summit, Kennedys Bush, Cass Peak) and no longer carried out in the three most disturbed sites (Ahuriri Valley, Otahuna Reserve, Tai Tapu) reducing the total trap number to 232. In both years, traps were checked at least twice during the year, approximately April/May and July/August. Any matai seeds were collected, numbered, bagged with silica gel and stored at -20°C.

In March 2010, additional seed traps were set up underneath male trees in Ahuriri Summit and Kennedys Bush in the same manner as the female seed traps. These traps served two purposes: to check whether male matai were producing any fruits (leaky dioecy) and to investigate whether males acted as foci for seed dispersal (i.e. if birds preferentially flew from female to male matai canopies). Five males were used in each site, each tree had three traps underneath it for a trap area of 0.16 m^2. Traps were checked for contents through to July 2010.

(a) Early stages of germination **(b)** Seedling at several months old

Figure 3.3: Matai seedlings exhibiting the split seed coat indicating a germinated seed

Transect lines of seed traps were set up running away from female matai trees in March 2010. Two sites were used, Ahuriri Summit and Kennedys Bush, with ten and eight fruiting female trees selected in each site respectively (fewer trees were used in Kennedys Bush due to the smaller matai population). Five traps were set up beneath each female, at distances of 5 m, 10 m, 15 m, 20 m and 25 m. Traps were checked for contents through to July 2010.

3.3.2 Ground seed fate

Matai seeds persist for years in the leaf litter and topsoil due to their thick endocarps, so it is possible to collect these seeds that accumulate on the ground. In January 2009, ground plots were set up under female matai trees in Ahuriri Summit and Kennedys Bush, five plots in each site. Plots were 0.5 m x 0.5 m; the plot area was marked out with pegs in each corner and a measuring tape perimeter, any seedlings counted, then all leaf litter and topsoil sifted and checked for matai seeds and seed coats. Collected seeds were classified into the following categories: whole (intact seed), half (clean split along the seed coat seam, i.e. the dehiscence plane, Salter, 2004) indicating a germinated seed, Figure 3.3), green, undispersed (flesh attached), rat and insect predated. The total number of "half" seeds collected per plot was divided by two to indicate original number of seeds. Three classes of seeds were collated to represent "germinated" seeds; half seeds (Figure 3.3), seedlings and seeds with a radicle emerging. Total numbers were multiplied by 4 to give counts per m^2 in order to relate numbers to grid ground plots (see below). A subset of seeds were cracked open and checked for seed contents (i.e. an intact embryo): 500 whole seeds, 200 rat predated and 200 insect predated seeds.

In January and February 2009, a further 51 1 x 1 m ground plots were set out in Ahuriri Sum-

mit on a rough grid across the entire field site. Because these traps were not directly under female trees, the larger plot size was chosen to account for smaller quantities of matai seeds. Contents were collected and categorised as described above for female plots. I recorded a GPS waypoint for each plot and used Hawth's Tools v3.27 (an add on for ArcMap) to calculate the distance of each ground plot from the nearest female matai tree.

To assess whether animals were removing matai seeds and fruit from the ground, I conducted a seed removal experiment in May 2008 in Ahuriri Summit and Kennedys Bush. Five locations were chosen in each site; in each location I placed five ripe fruits and five clean seeds. A small depression was cleared in the ground so the seeds could be re-located, approximating the "open ground" treatment of Moles and Drake (1999). Fruits and seeds were counted and checked for damage after 3, 7 and 10 days. The final check was planned for 14 days, but was prevented by heavy snowfall on the Port Hills. I used a quasibinomial glm to compare the proportion of seeds and fruits remaining at day 10 between seed state (clean/whole) and site. Human handling of seeds appears to affect the removal rate of seeds and fruits (Duncan et al., 2002; Wenny, 2002); because I took no precautions to prevent leaving human scent on the set-up, any removal results cannot be extrapolated to estimate predation or feeding rates on matai.

Following from the results of the seed removal study, I set up an infra-red video camera in Ahuriri Summit for five 24-hour sessions in June 2008. The five filming sessions were not consecutive; two sessions (5–6 June) were conducted before being interrupted by snow then the remaining three sessions were conducted six days later (12–15 June). Ten ripe matai fruit were placed in sight of the camera and time-lapse footage was recorded from the time the camera was set up (late morning) until approximately the same time the next day. Any remaining fruits were counted when the video was changed, and the video collected and analysed for animal activity.

3.4 Results

3.4.1 Seed traps

From traps under female trees, a total of 1,434 seeds and fruits were collected in 2009, and 1,650 in 2012 (Table 3.1). There was no difference in the mean number of seeds and fruits per tree caught between years (Table 3.2). In both years a small percent of trap catch was green, unripe fruit (2009 $\bar{x}$ = 5.9%, 2010 $\bar{x}$ = 3.9%). This indicates that few fruits failed late in development due to environmental causes such as high winds or drought; as these fruits failed and were not available to dispersers, I have not considered them further.

On average, 90% of fruits and seeds caught showed no evidence of either insect or rat pre-

dation (Table 3.1, Figure 3.4). The percentage of "clean" seeds caught decreased significantly between 2009 and 2010 but was not affected by site (Table 3.3). Although the percent of undispersed fruits appeared to increase from 2009 to 2010 (Figure 3.4), this was not significant nor was there a site effect (Table 3.4).

I found evidence for pre-dispersal predation by both rats and insects (where the insect was presumed to be *Heterocrossa iophaea*). Insect predation increased significantly between 2009 and 2010 and was not affected by site (Table 3.5). Given *H. iophaea* may feed on matai seeds earlier in the season (before the seed coat hardens), 2010 may appear to have higher predation rates because traps were set up earlier in the year. To check this, I repeated the quasibinomial glm using only "comparable" data, i.e. seeds collected at the same time of year; the percentage of insect predated seeds was still significantly higher in 2010 (Table 3.6). Rats are able to climb into matai canopies and predate seeds, but I found only a small amount of evidence for this (Table 3.1); there was no difference between years or sites (Table 3.7).

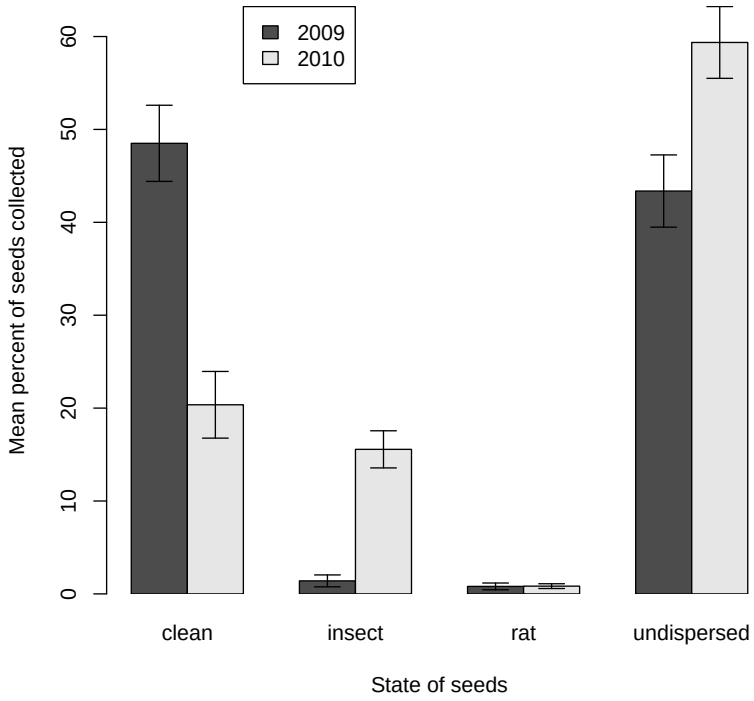


Figure 3.4: Condition of seeds caught in seeds traps under female matai canopy in 2009 and 2010. Error bars show $\pm$ 1 SE, means are calculated from ten trees, five from each of two sites.

Of the 356 grid traps set out in 2009, only 32 traps (9%) caught any matai seeds, for a total of 84 seeds (Table 3.8). I used a quasipoisson GLM to predict number of seeds caught by the distance from the nearest female matai tree; there was no relationship between distance and

Table 3.1: Mean percentage (± 1 SE) of fruits and seeds in each category caught under female matai trees (n = 5 trees per site).

Year	Clean	Insect	Rat	Undispersed	Total	Mean seeds per m^2
Ahuriri Summit						
2009	54.8 (2.6)	3.1 (1.0)	1.5 (0.7)	40.6 (3.5)	632	859 (183)
2010	18.7 (1.1)	19.0 (3.6)	0.6 (0.3)	61.6 (4.5)	404	520 (73)
Kennedys Bush						
2009	47.8 (7.7)	0 (0)	0.3 (0.2)	51.9 (7.7)	707	934 (215)
2010	23.4 (7.3)	13.5 (1.8)	1.1 (0.5)	62.0 (7.1)	1185	1543 (439)

Table 3.2: One-way ANOVA, mean number of seeds in female seed traps by year.

	DF	SS	MS	F-value	p-value
Year	1	2333	2333	0.20	0.66
Residuals	18	209378	11632		

number of seeds (Table 3.9, Figure 3.5), using the log of distance did not improve the fit (Table 3.9). Seeds were caught up to 130 m from a female matai, but 98% of seeds were caught within 55 m (Figure 3.5). In 2010, only 6 seeds were caught across 6 traps in Ahuriri Summit and Kennedys Bush (from a total of 232 traps across four sites), I have not considered these seeds further.

Seeds traps located under male matai canopies caught a total of 11 seeds in 2010. All of these seeds were dispersed (i.e. clean) indicating they had been moved there by birds. I found no evidence of leaky dioecy, as no undispersed fruits were caught in these traps. I used a quasipoisson GLM to assess the number of seeds caught in male traps and grid traps, predicted by distance to the nearest female matai and whether traps were under males or not. Because male trees had three traps beneath them while grid traps were individual, I used an offset function for glms which accounts for the difference in trap effort. Distance to the nearest female was a significant predictor of seed catch but being under a male was not significant (Table 3.10).

In total 76 seeds were caught along female transect trap lines, 55% of these were clean (through bird), 21% predated and 20% undispersed. No undispersed fruits were caught beyond 10 m, whereas 17% of the clean seeds went beyond 10 m (Table 3.11). Insect or rat predated seeds

Table 3.3: Quasibinomial GLM: proportion of clean seeds caught under female trees in two sites over two years.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			19	408.20		
Site	1	9.36	18	398.84	0.73	0.405
Year	1	129.53	17	269.31	10.13	**0.006**
Site:Year	1	47.00	16	222.30	3.67	0.073

Table 3.4: Quasibinomial GLM: proportion of undispersed fruits caught under female trees in two sites over two years.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			19	257.39		
Site	1	17.25	18	240.14	1.50	0.24
Year	1	12.30	17	227.84	1.07	0.32
Site:Year	1	35.56	16	192.28	3.09	0.10

Table 3.5: Quasibinomial GLM: proportion of insect predated seeds caught under female trees in two sites over two years.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			19	260.85		
Site	1	2.20	18	258.66	1.19	0.292
Year	1	208.69	17	49.97	112.96	$<$ **0.001**
Site:Year	1	19.42	16	30.55	10.51	**0.005**

Table 3.6: Quasibinomial GLM: proportion of insect predated seeds caught under female trees in two sites over two years – only data for comparable timing in the seasons.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			19	207.80		
Site	1	0.07	18	207.74	0.03	0.856
Year	1	153.22	17	54.52	74.95	$<$ **0.001**
Site:Year	1	19.91	16	34.61	9.74	**0.007**

Table 3.7: Quasibinomial GLM: proportion of rat predated seeds caught under female trees in two sites over two years.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			19	31.65		
Site	1	2.71	18	28.95	2.42	0.14
Year	1	0.08	17	28.86	0.07	0.79
Site:Year	1	6.80	16	22.06	6.08	**0.03**

Table 3.8: Matai seed catch from 356 traps set out across seven Port Hills field sites in 2009. N traps caught indicates the total number of traps that caught a seed, and mean seeds per trap is calculated from just these traps (i.e. does not include traps that = 0).

Site	N traps	N traps caught (%)	N seeds caught	Mean seeds per trap	Mean distance from nearest female (m)
Ahuriri Summit	65	11 (17)	21	1.9	29.21
Ahuriri Valley	64	3 (5)	8	2.7	54.67
Cass Peak	10	1 (10)	2	2.0	6.30
Kennedys Bush	57	5 (9)	8	1.6	33.05
Omahu Bush	40	1 (3)	1	1	39.75
Otahuna Reserve	70	3 (4)	10	3.3	10.13
Tai Tapu	50	8 (16)	34	4.3	21.58

were only caught in traps 5 m away from females, i.e. still underneath the canopy. Insect predation (20%) was more common than rat predation (1%).

Table 3.9: Quasipoisson GLM with number of matai seeds caught in grid traps in 2009 predicted by distance from nearest female matai tree.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
Model 1						
NULL			31	79.212		
Distance	1	1.49	30	77.72	0.34	0.56
Model 2						
NULL			31	79.212		
Log (Distance)	1	0.0003	30	79.21	0.0003	0.99

Table 3.10: Quasipoisson GLM with offset function: number of seeds caught per trap predicted by distance from the nearest female and whether a trap was under a male matai or not.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			101	117.88		
Distance	1	12.86	100	105.03	4.44	**0.04**
Male	1	9.82	99	95.21	3.39	0.07

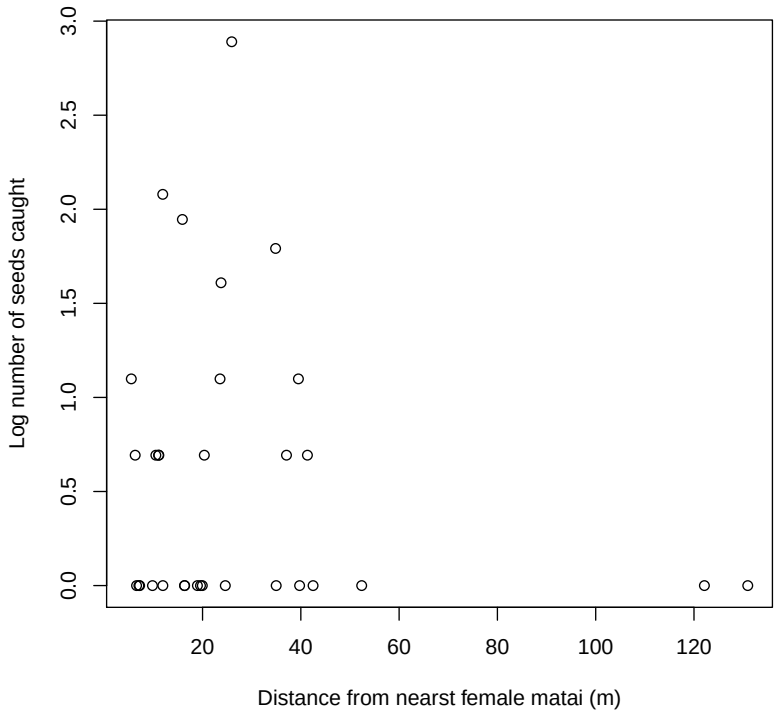


Figure 3.5: Distance away from the nearest female matai tree of seeds caught in traps across sites in 2009.

Table 3.11: Total seeds collected in 2010 from seed traps set along transects from fruiting female trees combined for both Ahuriri Summit and Kennedys Bush, % through a bird indicates clean seeds with flesh removed.

Distance	Total	Clean	Green	Insect	Rat	Undispersed	% through bird
5 m	63	31	3	15	1	13	49
10 m	6	4	0	0	0	2	66
15 m	3	3	0	0	0	0	100
20 m	3	3	0	0	0	0	100
25 m	1	1	0	0	0	0	100
Total	**76**	**42**	**3**	**15**	**1**	**15**	

3.4.2 Ground seed fate

The average density of seeds collected from ground plots underneath females was 4971 per m^2 ($\pm$ 849 1 SE). By taking this average number of seeds and comparing it to the average number of seeds caught in traps under those same female trees ($\bar{x}$ = 915/m^2), I estimated that the ground plots reflect 5–6 years of matai seed fall. Of the subset checked for contents, none of the rat or insect predated seeds had the remains of an embryo; however, 14.6% of the whole seed subset still had an embryo when they were opened. I found no correlation between the number of seeds collected from under female ground plots and the number of seeds caught in traps under those same trees in 2009 (r = -0.04, df = 8, p = 0.91) and 2010 (r = 0.04, df = 8, p = 0.90). This suggests that any differences in seed output in a given year may be evened out across multiple fruiting years.

Green fruit made up less than 1% of the fruits and seeds collected, reflecting the seasonal timing that plots contents were gathered as green fruit had not yet begun falling. Of the seeds fallen beneath female trees, a small percentage showed signs of rat predation (Table 3.12); this is likely to be an underestimate of rat predation (see Discussion). Insect predated seeds made up an average of 8% of the under-female ground plot seeds (Table 3.12). This is within the range of insect predation found in seed traps (2009 $\bar{x}$ = 1%; 2010 $\bar{x}$ = 16%), perhaps indicating an averaging out of insect predation rates across multiple years of seed fall. "Germinated" seeds made up an average of 18% of seeds ground plot collections, although seedlings only occurred at a rate of 3.2/m^2.

Table 3.12: State of seeds per 1 m^2 collected from ground plots underneath female matai trees, mean percent ($\pm$ 1 SE).

Site	Whole	Insect	Rat	Germinated	Undispersed	Total
Ahuriri Summit	64.3 (2.3)	7.1 (1.5)	4.9 (0.5)	19.6 (1.9)	3.9 (1.1)	31,170
Kennedys Bush	66.7 (3.0)	9.1 (0.8)	4.2 (0.3)	16.5 (2.3)	3.5 (2.1)	18,540

I measured the distance between each grid plot and the nearest female matai ($\bar{x}$ = 38.5 m $\pm$ 4.6 SE) (Figure 3.6). Using a quasipoisson glm between the number of seeds per m^2 and distance from the nearest female matai, I found a significant negative slope with total number of seeds decreasing with log distance (Table 3.13). Insect predated seeds were found up to 24 m from a female matai, while rat predated seeds were found up to 47 m away. The discovery of insect predated seeds away from female trees suggests that either birds or mammals can still consume

Table 3.13: Quasipoisson GLM number of seeds found in ground plots predicted by log(distance) from nearest female matai.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			50	4195.3		
Log(distance)	1	1066.1	49	3129.1	6.09	0.02

a seed that has been predated, thus moving them away from the female tree where predation occurred.

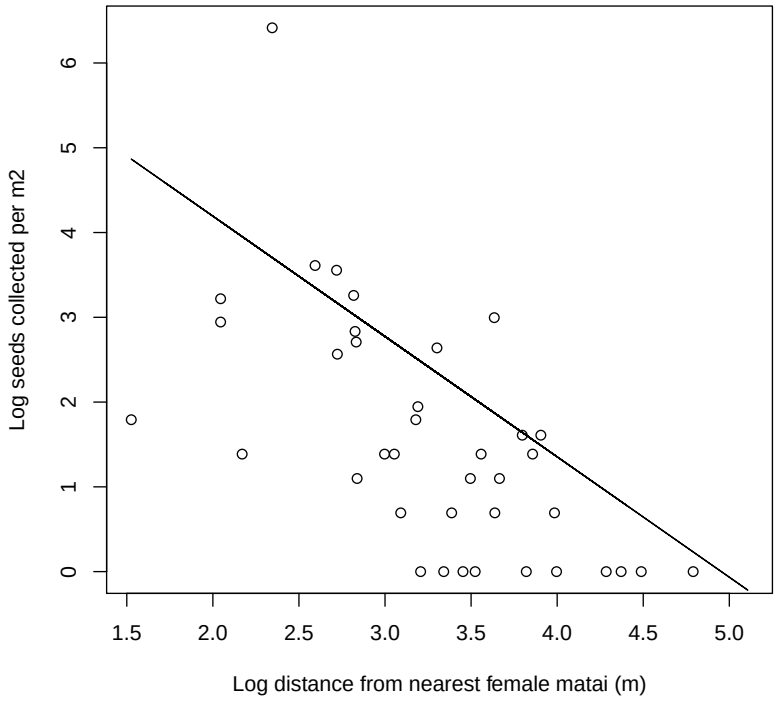


Figure 3.6: Log-log plot of matai seeds collected in 1 m^2 ground plots, fitted values from a quasipoisson glm. Number of seeds caught decreased significantly with distance from the nearest female matai (Table 3.13).

The average percent of seeds collected in plots under females in each seed category appeared to differ from that collected away from females (Figure 3.7) but generalised linear models did not find significant differences (Table 3.14). A binomial glm takes the sums from all plots combined to calculate predicted percentages, rather than taking a percentage for each plot then averaging across plots, as I have for Figure 3.7. The lack of significance between female and grid plots (Table 3.14) may be driven by variability between plots which is masked by taking an overall

percentage. Of the 51 plots away from females, only five (9.8%) contained rat predated seeds, a further five contained insect predated seeds while germinated seeds were present in 32 (62.8%) plots. Due to the low number of plots containing rat or insect predated seeds, I cannot evaluate the effect of distance from female on predation rates. There was no evidence that the proportion of germinated seeds changed significantly with distance from female ($F_{1,35}$ = 0.09, p = 0.77) or total number of collected seeds ($F_{1,34}$ = 1.22, p = 0.27).

Table 3.14: State of seeds collected from ground plots under and away from female matai trees, Ahuriri Summit; results from quasibinomial glms with location (under/away) as a predictor. Mean percentages are calculated as for a quasibinomial glm, e.g. sum of all germinated seeds across all plots divided by total seed count, rather than percentage calculated per plot and then averaged as for Figure 3.7.

Category	Under female mean (%)	Grid mean (%)	df	F-value	p-value
Germinated	19.35	26.76	1,41	3.51	0.07
Insect predated	6.25	1.76	1,41	3.65	0.06
Rat predated	4.80	3.96	1,41	0.54	0.47
Whole	63.95	67.51	1,41	0.60	0.44

Whole fruits from the seed removal study began to be removed by the first check (3 days) (Figure 3.8). Whole fruits were removed significantly faster than clean seeds, but there was no difference between sites (Table 3.15). Removed fruits and seeds were not recovered, so I could not identify what animal had removed them.

Infra-red videoing recorded 96 hours of footage across the five sessions of filming; the first two sessions did not record a full 24 hours of footage due to battery issues. Of the 96 hours of footage, 39 hours were during daylight hours and 57 hours were over-night. For four of the five sessions, no fruit was removed from the trial set up. The second video (5–6 June 2008) filmed fruit removal by a single song thrush. In the morning, four of the ten fruits were remaining. Video analysis found the song thrush visited the trial set up at 12:30 (two hours after the camera

Table 3.15: Quasibinomial GLM seeds removed, status = clean, flesh.

Predictors	df	Deviance	Residual df	Residual deviance	F-value	p-value
NULL			19	75.16		
Site	1	0.18	18	74.98	0.08	0.783
Status	1	23.92	17	51.05	10.14	**0.005**

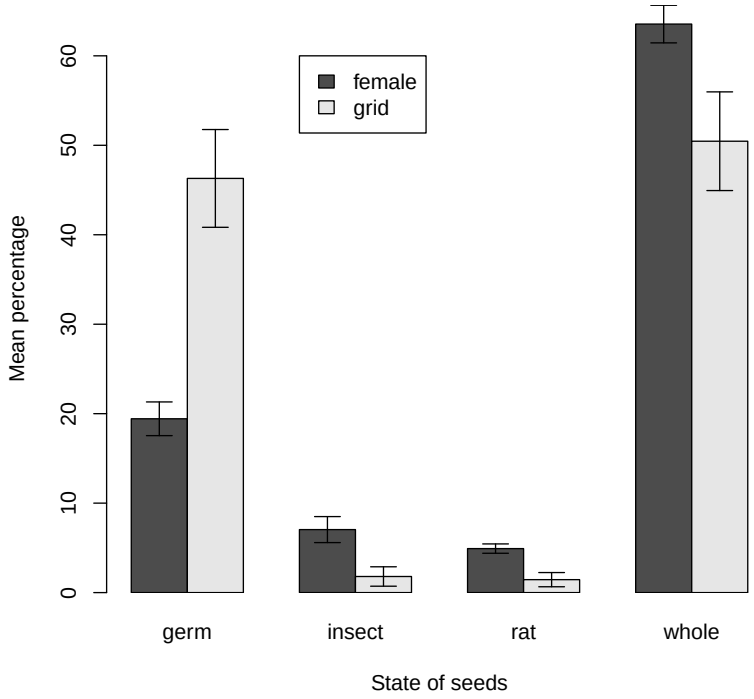


Figure 3.7: State of seeds collected from ground plots under and away from female, Ahuriri Summit 2009. "Germinated" includes seedlings, seeds that were in the process of germinating, and seeds that had split in half indicating previous germination. Error bars are $\pm$ 1 SE.

was set up) and again at 14:50. In the first visit, the bird spent a minute in frame and consumed 5–6 fruits (though appeared to drop one of them); when it returned, it ate a single fruit and left within one minute of arriving. There were no more visits by the bird in this filming period. In early evening (17:00–18:00) a mouse entered the frame but did not manipulate the remaining fruits. In two more videos (13–14 June; 14–15 June) a song thrush appeared in frame in early afternoon, but did not feed on any of the matai fruit set out. Its movements, and particularly the perch it used when entering the area, suggested that it was possibly the same bird filmed feeding on the trial fruits on 5–6 June.

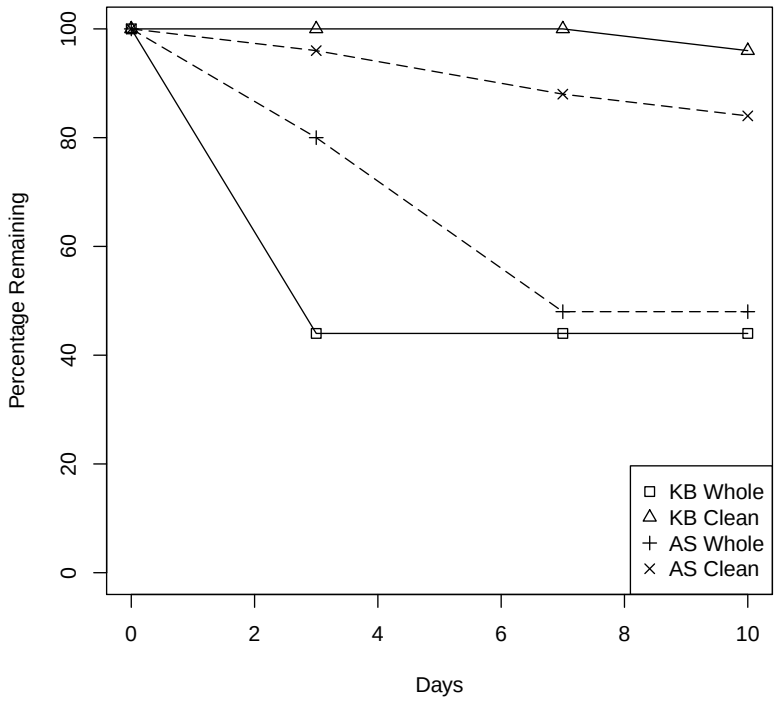


Figure 3.8: Removal rate of whole fruits and clean seeds from seed removal set ups. A greater proportion of whole fruits were removed compared to clean seeds. Legend indicates site (AS = Ahuriri Summit, KB = Kennedys Bush) and whether seeds were whole or clean.

3.5 Discussion

I measured seed dispersal service in fragmented sites using the proxy of percent "clean" seeds caught beneath female trees. This will under-estimate actual dispersal because it cannot measure seeds that are moved away from the parent tree, but in the absence of removal data (e.g. by measuring total crop and quantifying how much is taken which is very difficult in an emergent canopy tree like matai), such proxies are the best we have.

Without an explicit comparison to intact forest, it is difficult to ascertain whether the "through bird" levels I measured on the Port Hills are indicative of matai as a whole, or if this represents reduced service in fragmented environments. However, we can see that the removal rates I measured are in line with studies found elsewhere, and while they may not reach the heights experienced pre-disturbance, such rates may be sufficient to maintain dispersal service. In my study sites, up to 50% of the seeds collected under female canopies had passed through a bird; this does not account for seeds passed through a bird and deposited away from the maternal parent (i.e. dispersed). While acknowledging this limitation, the removal rates found here in matai are relatively low to those measured elsewhere. In Kaikoura, an average of 50% of seeds collected under fruiting *Beilschmedia tawa* trees had been through a bird, ranging from 13–80% between years (Kelly et al., 2010), while two mistletoes (*Alepis flavida, Peraxilla tetrapetala*) in Craigieburn experienced high fruit removal rates (92–98.5% of fruits) (Kelly et al., 2004). The exotic *Berberis darwinii* also received high removal rates (60–95%) (Allen and Lee, 1992). Similarly high rates of fruit removal have been found in Spain (89–100%, Herrera, 1984) and Australia (84%, French et al., 1992). Whether the removal rates I found in matai are lower due to effects of fragmentation would require explicit comparisons with removal rates in intact forest; however, not all species receive high removal rates. In Spain, even though *Frangula alnus* was a targeted food resource for frugivorous birds, and these birds closely tracked the availability of *F. alnus* fruits, only 53% of seeds in traps had been consumed by a frugivore (Hampe, 2008). What is evident is that frugivores are visiting matai canopies in the fragmented sites on the Port Hills and are consuming a portion of the available fruit crop.

In the Port Hills study sites matai appeared to be an abundant fruit resource. In early March 2009, while I was setting out seed traps, I noticed a sharp increase in kereru abundance in Ahuriri Summit where several weeks earlier it was rare to see more than one bird a day. In March, up to a dozen kereru flocked about the site in the morning, and during the afternoon seemed to be feeding singly or in pairs and were frequently startled while I went about the business of setting out seed traps. As matai is the most common emergent canopy tree in Ahuriri Summit (Appendix A),

with an abundant fruit crop (mean 915 seeds/m^2), it likely forms a large food resource for frugivorous birds in the area over autumn. Although totara and kahikatea are both present in Ahuriri Summit, they are less abundant and I only saw one kahikatea fruit heavily over the years I spent working in the site. Additionally, across all seven sites matai was the most frequent seed found in seed traps. The only other species that I identified regularly were supplejack (*Ripogonum scandens*) and five-finger (*Pseudopanax arboreus*). *Fuchsia excorticata* is abundant in many of the Port Hills sites, but generally grows toward the edges of the fragments rather than the centres where the podocarps dominate.

Although monitoring bird abundance was not part of this study, my observations tentatively suggest kereru may be tracking food sources in the area, as they are known to do elsewhere in the country. Densities of kereru have been found to increase dramatically when kahikatea (Robertson and Hackwell, 1995), miro (Clout et al., 1986, 1991) or rimu (Spurr et al., 1992) fruits ripen. Tui have also been observed flocking to heavy kahikatea fruit crops (Beveridge, 1964). Clout et al (1986; 1991) found radio-tracked kereru made long distance movements between seasonal food sources, and suggested that individual birds may be consistent in their annual use of seasonal ranges. More recent advances in animal tracking technology, including satellite telemetry, will provide more data on these large scale movements (e.g. Powlesland et al., 2011). Such seasonal behaviour suggests that large podocarp fruit crops are important to kereru diet, and on the Port Hills matai forms a large portion of this fruit crop.

Additionally, if matai fruit is a favoured food source in these sites then this may enhance dispersal service despite being in forest fragments with presumed reduced frugivore abundance. This phenomenon has been seen in Kenya, where *Prunus africana* trees in fragments experienced marginally higher dispersal rates than those in intact forest, despite having lower frugivore species richness (Farwig et al., 2006). Given that this contradicts the usual findings of reduced seed dispersal service in disturbed sites (e.g. Cordeiro and Howe, 2003; Rodriguez-Cabal et al., 2007; Kirika et al., 2008) the authors suggest perhaps an impoverishment of food supply in these fragments lead to a particular attractiveness of *P. africana* fruits. If large fruit crops, such as that in matai, attract frugivores across a generous spatial scale, they may inadvertently enhance the service of other plants in the area. An interesting question would be whether ubiquitous species (e.g. *Hedycarya arborea*, *Melicytus ramiflorus*, *Pseudopanax arboreus*, *Ripogonum scandens*) exhibit changes in dispersal service depending whether they are in a fragment with a large fruit source (e.g. matai) or not.

Further study of such mechanisms in these forest fragments would require two additional aspects that I did not measure in this project: frugivore abundance and availability of alternative

food sources. A measurement of bird abundance across the season would test my impression of increased kereru abundance as matai fruit ripened, while availability and use of alternative food sources will indicate several things, including whether birds are tracking matai fruit in particular, whether other plant species receive similar dispersal service, or whether impoverishment of surrounding food sources leads to a reliance on matai crop in degraded fragments. European starlings are well known for their flocking behaviour, and may follow food sources in this way (Linz et al., 2007). I discuss this further in Chapter 4.

In both years of data collection, seed predation by *H. iophaea* made up a substantial proportion of seeds caught underneath female trees. The significant increase in predation between 2009 and 2010 (Figure 3.4), though difficult to analyse in a long-term sense, would seem to have implications for bird dispersal. Sullivan et al. (1995) found similar rates of insect predation in Ahuriri Valley: 24% from ground samples and 11% from seed traps. Presumably seeds that are predated, and thus do not ripen, would not be consumed by birds (although kereru have been observed feeding on green fruits of matai, Burrows, 1994*b*). The higher rate of insect predation in 2010 was correlated with a reduction in the proportion of seeds that had been passed through a bird, as we might expect if insect predation reduces the amount of ripe fruit available to birds. However, without experimental manipulation I cannot be confident about this connection. In any case, in 2010, of the stages measured, insect predation was the biggest barrier to fruits being available to dispersers, as neither late fruit failure nor rat predation affected much of the fruit crop. Beveridge (1973) attributed insect predation for the failure of matai crop across a seven year study in the North Island; despite the seeming importance of insect predation in my study sites as a barrier to fruit ripening, in the three years of study on the Port Hills (2008, 2009, 2010) I have not observed a complete failure of matai crop as described by Beveridge.

Pre-dispersal predation by rats affected a small proportion of matai fruits; although rats are known to climb into tree canopies and consume seeds prior to dispersal, they did not affect a large amount of matai fruit in my study sites. Beveridge (1964) found higher levels of rat predation on podocarp seeds in the canopy (80% of rimu, 26% of miro). Further rat predation occurs after fruits have fallen from the tree, with rat chewed seeds evident in soil samples. Taking predation rates from underneath female trees may underestimate overall rate as rats may remove seeds to "husking stations" before consumption (Campbell et al., 1984; McConkey, 2003). Even so, with some 80% of seeds collected from under-female ground plots showing no sign of damage from predators, this does contradict the assertion that for a nearby site "almost all *Prumnopitys* seeds at Ahuriri [Valley] are eaten by ship rats" (Burrows, 2006, p. 112). It is unclear why rat predation in my study sites, both in the canopy and on the ground, was lower than that observed

elsewhere. Predation on *Peraxilla* sp. flowers by caterpillars (*Zellaria maculata*) showed a significant relationship with latitude; predation increased at lower latitudes, which may in turn be driven by the distribution of a parasitoid wasp (*Campoplex* sp.) that affects *Z. maculata* (Kelly et al., 2008). An effect of latitude could explain the deviation from the observations of Beveridge (1964), or alternatively if rats show a reduced numerical response to altitude, the higher altitude at Ahuriri Summit (400-470 m a.s.l) compared to Ahuriri Valley (40–200 m a.s.l) may explain the observations of Burrows (2006). King (2005) describes ship rats as being less common at higher altitudes. If rats do move seeds away to husking stations, some of these seeds may escape predation; on Tiritiri Matangi Island, 38% of *Elaeocarpus dentatus* seeds found in husking stations were intact (Campbell et al., 1984).

Fruits that fall to the ground may be subject to predation, but they can also be available to legitimate seed dispersers. In this case, I filmed a song thrush removing matai fruit from the ground which would result in legitimate dispersal. On mainland New Zealand, where introduced mammalian predators are widespread, it is rare to observe native forest birds (e.g. kereru, tui) on the ground. Introduced birds however, such as blackbirds and song thrushes, regularly feed on the ground; O'Donnell and Dilks (1994) found that for 28% of blackbird feeding observations the bird was on the ground. As we have seen here, these birds may therefore disperse fruits unavailable to native birds. I have also found introduced feral pigs consume fallen matai fruits and disperse some intact seeds (see Chapter 5). Such dispersal behaviour by introduced species, while perhaps not completely substituting for native dispersers, may partially compensate for reductions in the native avifauna.

Gender dimorphism in plants is often considered in discrete terms, with species frequently defined as monoecious or dioecious when perhaps the true condition is for a continuum between various states (Lloyd, 1980). Further investigation into breeding systems of native species is uncovering some of this grey area. Karaka (*Corynocarpus laevigatus*), typically classed as monoecious (Allan, 1961) has recently been re-defined as gynodioecious (Garnock-Jones et al., 2007). Female trees bear flowers with anthers, but these anthers do not contain pollen (staminoides), meanwhile male trees bear flowers with fully developed ovules and set small amounts of fruit. Such a system can been seen as having constant females and inconstant males (Garnock-Jones et al., 2007). Kohekohe (*Dysoxylum spectabile*) exhibits the opposite change in knowledge; traditionally called dioecious, studies have found "males" can set small amounts of fruit (Braggins et al., 1999; Gardner, 2009; Burns, 2011). This inconstancy of one or the other sex is referred to as leaky dioecy, where occasionally an individual of a dioecious species produces either hermaphrodite flowers or flowers of the opposite sex (Venkatasamy et al., 2007). The in-

constancy is more often on males and appears to be common within island floras (Webb et al., 1999).

The typical explanation for the greater frequency of dioecious species on islands has been that they dispersed as monoecious or hermaphrodite species and later evolved dioecy (Venkatasamy et al., 2007). However, Bawa (1982) showed that Hawaiian genera for which this was assumed already existed outside of Hawaii as dioecious species. The presence of leaky dioecy, however, provides another explanation, by which a nominally dioecious species may occasionally be able to self-pollinate. Dioecy has implications for seed dispersal in that, compared to cosexual species, dieocious plants have only half as many individuals producing seeds. Even if dioecious females produced twice as many seeds as a cosexual species, the resulting seed shadow would be more dense than that of the cosexual species, potentially increasing competition among seedlings (Heilbuth et al., 2001). There must be a strong evolutionary benefit for dioecy to evolve, possibly in the form of avoidance of inbreeding, which in some long-lived plants has been found to strongly affect plant survival (Scofield and Schultz, 2006; Robertson et al., 2011).

These recent developments in the New Zealand native flora lend credence to prior observations that both miro and matai occasionally exhibit cones of the opposite sex, i.e. leaky dioecy (McEwen, 1988; Leathwick, 1984; Salter, 2004). Although I found no evidence for male matai trees producing occasional female fruit, this was based only on seed trapping under five males in a moderate fruit year and specific observations on the sexuality of trees could yield results such as that described above for karaka and kohekohe.

Assessing the spatial movement of dispersed seeds in this study is limited by the inability to use genetic markers to identify the maternal parent of dispersed seeds (see Chapter 2). Instead, I have used distance to the nearest female matai as a conservative estimate of dispersal distance, although this model is known to underestimate dispersal (Nathan and Muller-Landau, 2000). Using the "nearest-female" model severely limits the maximum dispersal distance possible to be measured, and in my study the greatest distance a seed was found from the nearest female was 130 m. Even with these restrictions, it is evident that within my Port Hills field sites matai seeds are being consumed and moved about by dispersal agents, most likely birds (see Chapter 4), at least tens of metres from the maternal parents. Evidence of germination can be found throughout the fenced reserves, indicating the availability of an ongoing cohort of young plants available for recruitment should the opportunity arise.

Chapter 4

Observations on avian dispersers of matai

View of matai canopy from the ground.

4.1 Abstract

I observed avian visitors to fruiting female matai canopies for 81 hours over three weeks in autumn, 2009. Six frugivorous bird species were observed feeding on matai fruit, although silvereyes were only seen feeding once. The other five species contributed differing amounts to the number of fruit feeding observations: kereru (50%), bellbird (25%), song thrush (12.5%), blackbird (7.9%), starling (2.6%). Bellbirds exhibited the highest visit rate per hour ($\bar{x}$ = 6.37) but a relatively short visit length ($\bar{x}$ = 110 secs). Kereru made less frequent visits ($\bar{x}$ = 2.39 per hour) but spent much longer in the tree on each visit ($\bar{x}$ = 488 secs). Blackbirds and song thrushes both made less frequent and shorter visits and thus spent less total time in trees than bellbirds and kereru. Starlings were only observed on a single day, which perhaps reflects the flocking behaviour of this species. Birds were less active during periods of high wind; generally birds visited matai canopies less frequently, but did not alter the length of visits. *Turdus* species (blackbird and song thrush) took longer to observe entering matai canopies, this did not appear to be due to "shyness" but rather reflects the lower abundance of these two species. The majority (89%) of species interactions involved a native bird as the aggressor; an exotic bird was never observed behaving aggressively toward a native species. Combined, the three exotic species contributed 24.3% of the fruit feeding observations in matai canopies; this is greater than has been found in other studies which gather data through mist-netting or walking transects. The lower abundance of the two *Turdus* species perhaps means that such methods under-estimate their contribution to frugivory.

4.2 Introduction

Globally, birds are important drivers of ecological processes (Sekercioglu, 2006) and species declines or extinctions may cause widespread effects for ecosystems (Sekerciolu et al., 2004). With respect to seed dispersal, loss of avian frugivores could lead to clumping of seedlings under parent plants, an inability to colonise new areas (and thus respond to environmental change) and a lack of gene flow between fragments or populations. Long-term demographic effects of such dispersal limitation may not be immediately evident due to long life cycles of some plants (Kelly et al., 2004), but could have large effects for future populations.

Bond (1994) detailed four conditions necessary for failure of a dispersal mutualism to affect plant abundance. Amongst these, he included that native mutualists provide insufficient visitation and introduced species do not substitute for the natives. Therefore, understanding which

bird species remove fruit and the degree to which they do so is important for monitoring dispersal mutualisms.

New Zealand has been a frequent case study for the impact of avian extinctions. With the arrival of humans, several frugivorous species were lost and others declined in range, leaving behind the "wreckage of an avifauna" (Diamond, 1984). Meanwhile, introduced avian species are now abundant across the country; in the latest survey of bird distribution, six of the ten most widespread birds were introduced species (Robertson et al., 2007). Whether New Zealand plants are suffering from failed dispersal mutualisms depends on what roles the remaining native birds fulfil, and whether introduced birds act as competent substitutes.

It seems logical to assume that animals will eat whatever food sources are available to them; in this case, that introduced birds (or mammals) would feed on available fruits regardless of whether those fruits are native or exotic. Seed dispersal differs from pollination in that plants cannot easily prevent an animal from eating their fruit, whereas elaborate flower morphology has evolved repeatedly to ensure the fidelity of a given animal or insect pollinator. Thus, we see few specialist dispersal systems, with fruits instead available to a wide array of animals (Wheelwright and Orians, 1982); in this case, naïve (i.e. exotic) animals may be able to consume fruits and act as seed dispersers (Kelly et al., 2004). In support of this, studies elsewhere are beginning to show evidence of naïve animals acting as seed dispersers of native plants (Foster and Robinson, 2007; Davis et al., 2009; Staddon et al., 2010), in some cases seeming to substitute for native mutualists (Kawakami et al., 2009; Chimera and Drake, 2010). On New Zealand's Three Kings Islands, native plants appear to have been largely reliant on introduced birds for dispersal until kereru were reintroduced in 1999/2000 (Bellingham et al., 2010).

Of the range of exotic birds in New Zealand, three are widespread and behave as major frugivores and seed dispersers in their native range: blackbird (*Turdus merula*), song thrush (*T. philomelos*) and European starling (*Sturnus vulgaris*) (Herrera and Jordano, 1981; Snow and Snow, 1988; Debussche and Isenmann, 1989; Rey et al., 1997; Fuentes et al., 2001; Hernandez, 2005; Linz et al., 2007). All three birds are of a medium size (21–25 cm long, 70–90 g), and should be able to swallow the majority of New Zealand native fleshy fruits (though not the largest fruit species, (Clout and Hay, 1989; Kelly et al., 2010)). However, previous research has suggested that introduced birds provide little dispersal service to native plants, instead feeding predominantly on exotic species (Williams and Karl, 1996; Kelly et al., 2006). As this conflicts with the above notion of generalised seed dispersal systems, I conducted observations on female matai canopies during the fruiting season to examine bird contribution to fruit removal. Specifically, I sought to answer the following questions:

1. Which birds feed on matai fruits on the Port Hills?
2. How long and how often do birds visit matai canopy?
3. How does wind affect bird behaviour?
4. How do native and introduced birds interact?
5. What is the relative importance of native versus introduced species to matai fruit removal?

4.3 Methods

Bird visitation to fruiting matai canopies was observed over three weeks in April 2009 in Ahuriri Summit and Kennedys Bush. Observation trees were chosen in each site (9 in Ahuriri Summit, 4 in Kennedys Bush) based on the extent of the canopy visible from the ground (i.e. not obscured by under-storey shrubs); each tree was observed between 1 and 7 times ($\bar{x}$ = 4.6). For each observation period one of the observation trees was chosen and the observer sat underneath the canopy with a pair of binoculars. At the start of the observation period, the time and weather conditions were noted and a stop-watch started. Any birds present in the canopy at the start of the observation period were recorded as being present at the start of the time period. Observations were made simultaneously by 2–3 observers, each on a different tree for a pre-determined period of time (1–2 hours). If a bird was still in the tree at the end of the observation period, it was recorded as such and its visit length was calculated as the end time being when it left the tree (i.e. an under-estimation of visit length).

When a bird was observed entering the canopy the following data were recorded: a species identity where possible, visit duration, the time since the start of the observation period and any particular bird behaviour (e.g. feeding, singing, preening). Species interactions were also recorded (e.g. bellbird chased a blackbird through the canopy). Where possible, a bird was followed for the entire time it was in the canopy, with an entry time, any particular behaviour, and exit time recorded. However, due to observation difficulties (several birds in the tree at once, areas of the canopy out of sight) entry and exit times were not always exact. For instance, a bird may not have been observed entering a tree and only spotted already *in situ*; in this case, the time the bird was observed was recorded as the entry time. In other cases, a bird may have left the tree without being seen; in this case, the exit time was recorded as the last time the bird was seen. A further complication occurred when more than one bird of a species was in the canopy at once, and it was unknown which bird was arriving or exiting. Where this occurred, the first bird to arrive was assumed to be the first bird to leave. Overall, whenever there was a question

about how long a bird spent in the observed tree, the most conservative (i.e. shortest) estimate of visit length was used. I calculated visit rates as the number of species A in observation period B, divided by the length (in minutes) of period B, multiplied by 60 to give visits per hour. Observations were not made in rain. Whenever the weather changed to rain or high wind and birds were not present during an observation period, observations were halted for that day.

Because of the nature of matai, with its tall trunk and emergent canopy, we were sometimes unable to record complete data on fruit visitation. Ideally, an empirical analysis would record fruit removal in order to determine the relative proportion of fruit consumed by each disperser. Due to the high canopy, and the back-lit view (see frontispiece, this chapter), we could not gather this type of data for matai visitors. Matai fruit tends to ripen on the outer branches of the canopy (pers. obs.) so we were able to identify feeding visits when birds moved to these outer branches to feed. However, we still could not see well enough to count the number of fruits removed. Instead, we recorded visit length, noted whenever a species was observed feeding on matai and named any visit that included feeding observations as a "feeding visit", regardless of how many fruits were consumed.

A further complication caused by the high canopy was the difficulty we had distinguishing the two *Turdus* species from one another. When a species identification was not obtained, we recorded the bird visitor as an unidentified *Turdus*. Of 224 *Turdus* observations, 67 of these were not identified to species (29.9%). Only two feeding visits by a *Turdus* was unidentified to species, suggesting a bird was likely to be identified when feeding on fruit. Leaving these unidentified data out would overestimate the proportion of time both *Turdus* species spent feeding on matai fruit as I would be removing primarily non-feeding data. Wherever a table or figure refers to "*Turdus*", I have analysed total numbers, including blackbirds, song thrushes and unidentified *Turdus*.

During observations, we noted that it appeared to take longer after starting observations before the first blackbirds and song thrushes were seen entering the canopies compared to bellbirds and kereru. I hypothesised that perhaps these birds were "shyer" and were scared away from the trees when we arrived to begin observing. To test this hypothesis, I calculated the average time to first observation for each of the four main frugivores. That is, in each observation period I recorded the first observation of each species, and averaged across all observation periods. Additionally, I calculated the mean number of birds seen across observation periods as a measure of species abundance.

Table 4.1: Bird observations in matai canopies in Ahuriri Summit and Kennedys Bush, April 2009. Feeding visits consist of any visit in which feeding on matai fruit was observed, and contribution to frugivory gives the percentage of the 152 feeding observations by each bird species.

Species	Obs periods present (n)	All visits	Feeding visits (% of all visits)	Contribution to frugiviory (%)
Kereru	39	204	76 (37)	50.0
Bellbird	51	528	38 (7)	25.0
Song Thrush	23	94	19 (20)	12.5
Blackbird	28	63	12 (19)	7.9
Unidentified *Turdus*	13	67	2 (3)	1.3
Starling	7	20	4 (20)	2.6
Silvereye	18	99	1 (1)	0.7
Grey Warbler	16	33	0 (0)	0
Fantail	14	30	0 (0)	0
Total	**55**	**1138**	**152**	**100**

4.4 Results

Across the three weeks of the observation study, we observed on 10 days and gathered 81 hours of data across 55 observation periods. We observed for 7 days in Ahuriri Summit, gathering 56 hours of data, and for 3 days in Kennedys Bush, for 25 hours of data. Eight bird species were observed visiting matai canopy: five natives (bellbird, fantail, grey warbler, kereru and silvereye) and three exotics (blackbird, song thrush and starling).

4.4.1 Frugivory

Six bird species were observed feeding on matai fruit: kereru, bellbird, silvereye, blackbird, song thrush and starling (Table 4.1). Silvereyes were only observed feeding once, with a second possible sighting that did not have a confirmed species identification. Starlings were only observed on a single day and on that day a group was observed feeding on matai fruit; this fits with the flocking behaviour of starlings (see Discussion). The other four species (kereru, bellbird, blackbird, song thrush) were present in both sites throughout the observation period and regularly fed on matai. Other species recorded visiting matai were fantails (*Rhipidura fuliginosa*) and grey warblers (*Gerygone igata*). Both these species are small insectivores and were not observed

Table 4.2: Length and rate of visitation by main frugivores to fruiting matai trees. Superscript letters denote significant differences from TukeyHSD tests.

Species	Mean visits per hour ± SE	Mean visit duration (secs) ± SE
Bellbird	6.37 (0.60)^{A}	110 (6)^{A}
Kereru	2.39 (0.43)^{B}	488 (49)^{B}
Blackbird	0.77 (0.15)^{C}	150 (32)^{A}
Song thrush	1.05 (0.39)^{C}	352 (63)^{B}
Unidentified *Turdus*	0.78 (0.30)^{C}	80 (12)^{A}

feeding on matai fruit; I do not consider them further.

Of the four common frugivores, kereru spent the greatest proportion (37%) of their visits feeding on matai fruit, followed by blackbirds and song thrushes (Table 4.1). Bellbirds spent the smallest proportion of their visits feeding on matai. I calculated a contribution metric for each frugivore species as, of the total number of feeding visits, how many were made by each bird species (Table 4.1). Kereru contributed half of all feeding visits. Bellbirds, despite the small percentage of their visits that consisted of feeding, due to their high overall visitation contributed the next largest percentage of feeding, with song thrushes and blackbirds following.

4.4.2 Visit rate and duration

I averaged visit rates per hour across all observation periods (Table 4.2); visit rates exhibited positive skew, so I log-transformed the data with an offset of 0.1 (due to the prevalence of zeros in the data, i.e. no visits by a bird species during an observation period). A one-way ANOVA found a significant difference in visit rate between species (Table 4.3), so I used a TukeyHSD test to assess species differences. Bellbirds had the highest visit rate, significantly higher than kereru ($p < 0.001$), song thrushes ($p < 0.001$) and blackbirds ($p < 0.001$). Kereru, with the next highest visit rate, was significantly higher than song thrushes ($p < 0.001$) and blackbirds ($p = 0.002$). There was no significant difference between song thrushes and blackbirds ($p = 0.99$). Unidentified *Turdus* had significantly lower mean visit rates than bellbirds ($p < 0.001$) and kereru ($p < 0.001$) but showed no difference from song thrushes ($p = 0.56$) or blackbirds ($p = 0.27$). Therefore, bellbirds exhibited the highest mean visit rate per hour followed by kereru, with no differences in visit rate between blackbirds, song thrushes or unidentified *Turdus*.

The time birds arrived and left observation trees was recorded, thus allowing a calculation of visit length for every avian visitor (Table 4.2). I removed six observations that had “zero”

Table 4.3: One-way ANOVA testing visit rate against species.

	DF	SS	MS	F-value	p-value
Species	4	325.21	81.30	39.63	< 0.001
Residuals	270	553.90	2.05		

Table 4.4: One-way ANOVA testing visit length against species.

	DF	SS	MS	F-value	p-value
Species	4	220.41	55.10	25.96	< 0.001
Residuals	939	1993.51	2.12		

length. Visit lengths showed positive skew, so I log-transformed length and conducted a one-way ANOVA between visit length and bird species; there was a significant effect of species (Table 4.4) so I used a TukeyHSD test to examine which species differed. Kereru had the longest mean visit length, significantly longer than bellbirds ($p < 0.001$) and blackbirds ($p < 0.001$), but not different from song thrushes ($p = 0.49$). Song thrushes had significantly longer visits than blackbirds ($p = 0.007$) and bellbirds ($p < 0.001$). There was no significant difference between bellbirds and blackbirds ($p = 0.99$). Unidentified *Turdus* had significantly shorter visit lengths than kereru ($p < 0.001$) and song thrushes ($p < 0.001$), but showed no difference to bellbirds ($p = 0.85$) or blackbirds ($p = 0.95$). Therefore, kereru and song thrushes had the longest mean visit lengths and there was no difference between bellbirds, blackbird or unidentified *Turdus*.

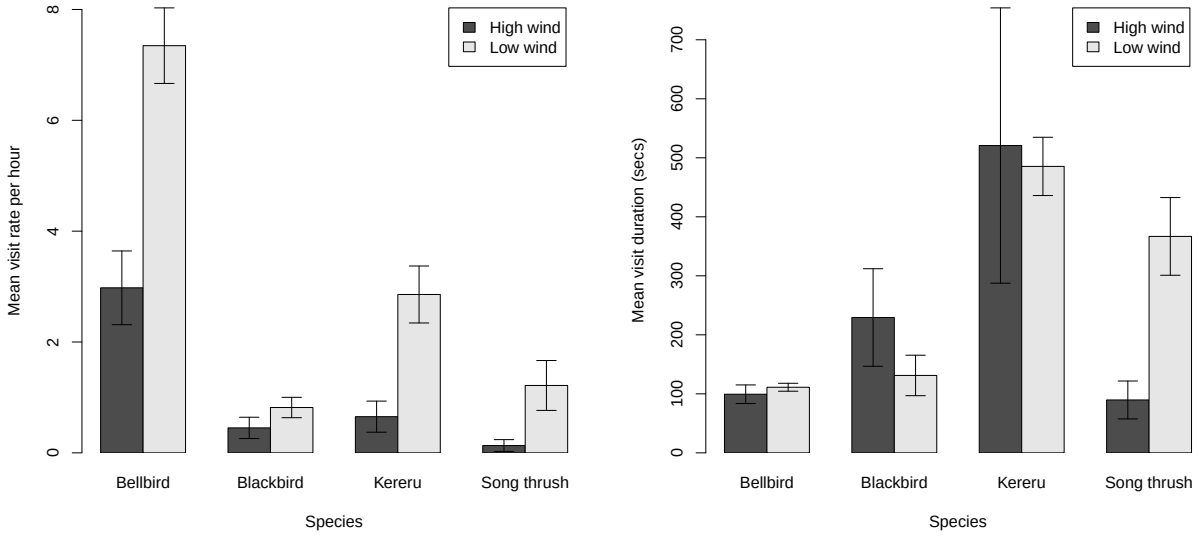
4.4.3 Effect of weather on visitation

Inclement weather appeared to have a significant effect on bird behaviour. We did not observe in rain, but occasionally we observed on days of high wind. In such weather, we observed that birds seemed to spend time lower in the forest strata, and almost totally avoided the emergent canopies of matai. If the wind died down during an observation period, we would notice birds becoming more active and soon returning to the observation trees. Of the 81 hours of collected data, 20:37 (25%) were classified as high wind, with the remaining 60:42 (75%) as low wind. To test the impact of wind, I used ANOVAs to assess the effect of wind (high/low) on visit rate, again using log(rate) with an offset of 0.1 (Table 4.5, Figure 4.1). Visit rate of bellbirds, kereru and song thrushes all decreased significantly in periods of high wind; blackbirds and unidentified *Turdus*

Table 4.5: Mean visits per hour in relation to high and low wind; ANOVAs on log(visit rate + 0.1) predicted by weather.

Species	Low wind (SE, n)	High wind (SE, n)	df	F-value	p-value
Bellbird	7.35 (0.68, 455)	2.98 (0.67, 69)	1,62	17.00	$<$ **0.001**
Kereru	2.86 (0.51, 190)	0.65 (0.28, 14)	1,62	10.20	**0.002**
Blackbird	0.82 (0.18, 51)	0.45 (0.19, 12)	1,62	2.13	0.15
Song thrush	1.22 (0.45, 89)	0.13 (0.11, 5)	1,62	7.55	**0.008**
Unidenfitifed *Turdus*	1.02 (0.42, 63)	0.11 (0.08, 4)	1,62	2.24	0.14

also decreased, but not significantly.



(a) Visit rate per hour

(b) Visit length (mins)

Figure 4.1: Mean visit rate and length of each of the main frugivores, separated into high and low wind. Error bars are $\pm$ 1 SE

I performed the same analysis on visit length of each frugivore species; as previously for visit length, I used log(length) to account for postive skew and removed six observations with zero length (i.e. the bird was recorded but did not stop in the canopy). None of the species differed in length of visit between the weather categories (Table 4.6, Figure 4.1). The majority of fruit feeding visits were made during calm weather (93%); kereru in particular seemed to drastically change their feeding behaviour during periods of high wind, only once was a kereru observed feeding during high wind (1% of feeding visits). This shows that birds did not change the length

Table 4.6: Mean visit duration (seconds) of each bird species related to high and low wind; ANOVAs on log(visit duration) predicted by weather.

Species	Low wind (SE, n)	High wind (SE, n)	df	F-value	p-value
Bellbird	112 (7, 451)	99 (16, 69)	1,518	1.36	0.24
Kereru	488 (50, 189)	521 (233, 14)	1,201	0.04	0.85
Blackbird	131 (34, 50)	229 (83, 12)	1,60	0.03	0.87
Song thrush	367 (66, 88)	90 (32, 5)	1,91	0.85	0.36
Unidentified *Turdus*	77 (12, 62)	146 (64, 4)	1,64	2.50	0.12

of time they spent in matai during windy weather, but most species did visit less frequently. Such data suggest, perhaps to the relief of field ecologists, that in order to measure fruit removal from high in the canopy it is more important to make observations in low wind conditions, when most of the feeding occurs.

4.4.4 Time to first observation and abundance

For each observation period, the time until each species was first observed was calculated (Table 4.7, Figure 4.2). In this instance, I analysed combined *Turdus* data ($\bar{x}$ = 21:13 $\pm$ 3:06) because the combined mean was lower than the mean of blackbirds ($\bar{x}$ = 24:43 $\pm$ 4:29) and song thrushes ($\bar{x}$ = 30:04 $\pm$ 5:13), indicating that excluding unidentified *Turdus* observations over-estimated the first time to see one of either species. There was a significant difference between species in the mean time until first observation (ANOVA $F_{2,125}$ = 4.47, p = 0.01); post-hoc analysis using a TukeyHSD test showed that bellbirds were significantly faster to arrive in trees than the combined *Turdus* (p = 0.01), but there was no difference between bellbirds and kereru (p = 0.10) or kereru and *Turdus* (p = 0.75).

Given that the length of observation periods differed, and observational studies frequently have relatively short observation times (cf. my observations ranged up to two hours in length) I calculated how many times we would have missed some of these common frugivores had we observed for shorter times (Table 4.7, Figure 4.2). Had we only observed for 15 minutes, half of the time we would not have seen either kereru or a *Turdus* species, though bellbirds would have appeared over two-thirds of the time. Although these percentages increased if I increased the observation time to 30 minutes, bellbirds and *Turdus* did not reach 100% until observations of 60 minutes, while kereru took over 80 minutes to reach 100%. This suggests that shorter ob-

Table 4.7: Time to first observation of each frugivore species and cumulative percentage of sightings which would have been made given specified observation lengths. Superscript letters indicate significant differences from a TukeyHSD test.

Species	n	Mean time (mins:secs)	SE	15 mins	30 mins	60 mins
Bellbird	49	$10{:}52^{A}$	2:04	82%	90%	100%
Kereru	38	$17{:}40^{AB}$	3:29	54%	79%	97%
Turdus	38	$21{:}13^{B}$	3:06	50%	68%	100%

servation periods may skew the data towards bellbirds; this may have implications for methods used to gather feeding data (see Discussion).

This, however, does not answer the question of whether some birds are shyer than others. To investigate further, I plotted mean visit rate of each species across the length of observations (Figure 4.3). Although all species seemed to decrease across observation length, this is likely an artefact of smaller sample sizes in longer observation times. I used paired t-tests to look for differences between the mean visit rate of each species in the first 15 minutes and the second 15 minutes of observation (i.e. 0–15 minutes versus 15–30 minutes of each observation period). There was no difference for bellbirds ($t = 1.68$, $df = 54$, $p = 0.10$), kereru ($t = 0.61$, $df = 54$, $p = 0.55$) or *Turdus* ($t = -0.68$, $df = 54$, $p = 0.50$) indicating that none of the species showed lower visit rates at the beginning of observation periods, the "shyness" that I had hypothesised.

Bellbirds were more abundant in trees than kereru or either *Turdus* species (Table 4.2). This is probably why bellbirds have a lower time to first observation (Table 4.7); they are so abundant that they are frequently seen in trees and thus observers did not have to wait long to see one.

These data show us that the combination of visit rate and visit length determines the total amount of time a bird species is present in a tree, which affects its observability. While bellbirds made significantly more visits to matai canopy, their short visit length means they were not in the canopy for as long as kereru were, with their less frequent but much longer visit lengths. The two *Turdus* species appeared to be the least often observed due to their combination of less frequent visits (cf. bellbird) and shorter visit lengths (cf. kereru).

4.4.5 Species interactions

Of the 28 aggressive interactions observed between birds, in the majority of these (26) the agressor was a native bird (Table 4.8). On 13 occasions we saw kereru chasing each other, while seven

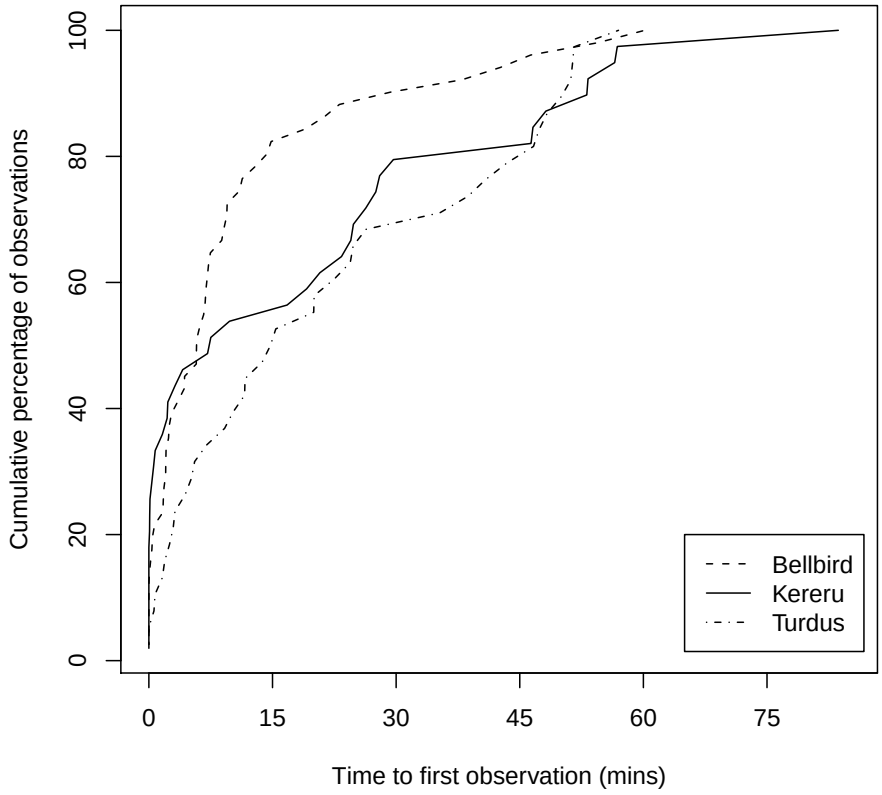


Figure 4.2: Cumulative percentage of time taken to observe the first individual of each frugivore species. Blackbirds and song thrushes are combined into "*Turdus*."

times we saw a bellbird chase another bellbird. Once a bellbird was seen chasing a silvereye. On one day an Australasian hawk (*Circus approximans*) was sighted circling over the tops of the native canopy, and we saw one kereru apparently startled by the hawk's presence. An introduced bird was never observed attacking or chasing a native bird, and overall only two observed interactions were instigated by an introduced bird, both times between blackbirds. However, on four occasions a native bird chased an introduced bird: twice we observed a bellbird chasing a blackbird, once a kereru chasing a blackbird and once a kereru chasing a song thrush. The bellbird–blackbird interaction is surprising since bellbirds (26–34 g) weigh much less than blackbirds (90 g). Due to the small count numbers in some cells (Table 4.8) I could not use a χ^2 test on these data; instead I used Fisher's exact test which allows for expected frequencies less than five but provides only a p-value with no associated test statistic (Crawley, 2005). There was a significant relationship betweeen species status (native/exotic) and aggressive interactions (p = 0.04, df = 1, FET). At least in this study area, introduced birds did not have negative social interactions with native birds, thereby we could conclude in this area that introduced birds do not have an effect

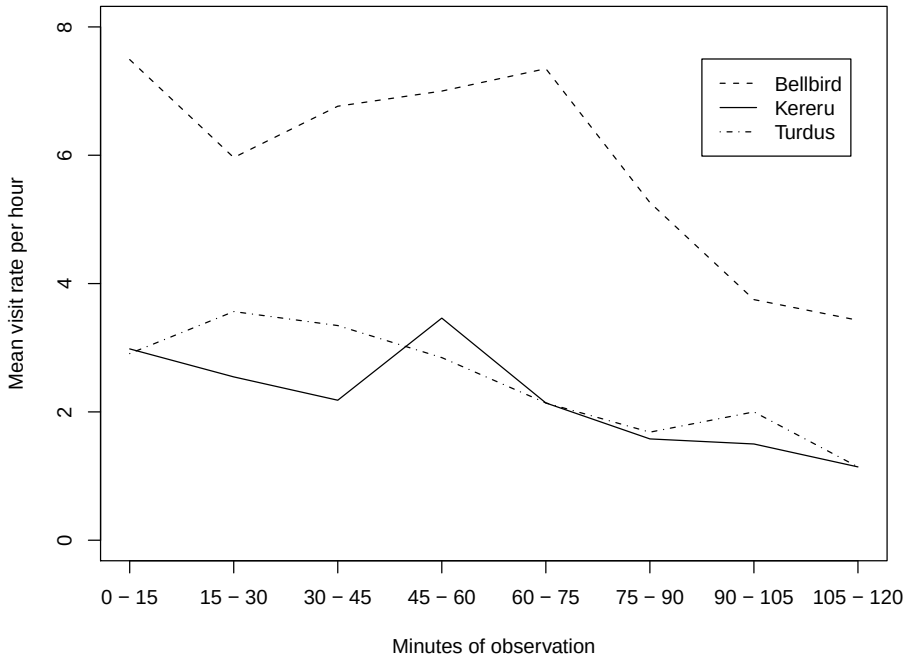


Figure 4.3: Mean number of birds seen in each 15 minute block of observation

Table 4.8: Avian species interactions observed in fruiting matai canopies.

Agressor \ Chased	Native	Introduced
Native	22	4
Introduced	0	2

on the behaviour, feeding or otherwise, of native birds.

4.5 Discussion

I have found a range of native and exotic birds feed on matai fruits within forest fragments on the Port Hills. Exotic birds are considered to contribute little to frugivory and dispersal of native plants in New Zealand (Williams and Karl, 1996; Kelly et al., 2006) and blackbirds have been implicated in the spread of fleshy-fruited weeds (Williams, 2006). However, in two Port Hills field sites I found blackbirds and song thrushes contributed one-fifth of observed feeding visits to matai fruits. Kereru performed the majority of frugivory, but the two *Turdus* species spent 15% of their visits to matai feeding on its fruit, twice that spent by the native bellbird. Bellbirds made up for their low feeding rate through sheer abundance and high overall visitation.

Frugivory studies in New Zealand have taken several different approaches to quantifying bird-fruit data; from single bird-multiple plant observations (e.g. Murphy and Kelly, 2001; Campbell et al., 2008; Emeny et al., 2009), multiple bird-multiple plant (e.g. O'Donnell and Dilks, 1994; Williams and Karl, 1996; Burns, 2012), and multiple bird-single plant (e.g. Kelly et al., 2006, this study). The method I used in this study, long observations on a single plant species, has not often been used in New Zealand. This method allowed for the inclusion of less frequent visitors (blackbirds and song thrushes) and observing for several weeks allowed the observation of itinerant visitors (i.e. starlings). These three exotic birds accounted for 24.3% of the fruit feeding visits to matai canopies.

There are several possible reasons, perhaps interacting, that may explain the higher rate of exotic birds feeding in my study. One is the numerical unimportance of silvereyes as dispersers of matai. Although matai seeds have been recovered from silvereye faeces (Williams and Karl, 1996), it appears that matai fruits fall on the size limitation of silvereye gape (Kelly et al., 2010). Thus, I only observed one definite occurrence of a silvereye feeding on matai. Other studies have found silvereyes to be among the most numerous fruit feeders (e.g. O'Donnell and Dilks, 1994; Williams and Karl, 1996; Kelly et al., 2006) so perhaps the relative importance of blackbirds and song thrushes is elevated in the absence of silvereye feeding. If this is the case, this is relevant to the dispersal of larger-seeded species for which silvereye cannot be a major disperser (Clout and Hay, 1989; Kelly et al., 2010) and perhaps indicates a greater possible importance of exotic birds for dispersal of such species.

A second possible explanation may be that, as I discussed in Chapter 3, matai appears to be a substantial food source on the Port Hills in autumn for frugivorous birds. If there is a paucity of other food sources in the area, we might expect that all frugivorous birds focus on matai in this area during autumn, perhaps explaining this increase in exotic feeding.

Finally, the nature of blackbirds and song thrushes (quiet, less abundant) may result in an under-representation in surveys of frugivory. Both O'Donnell and Dilks (1994) and Williams and Karl (1996) recorded a disparity in the relative numbers of different frugivores. Silvereyes vastly outnumbered all other species observed in both studies, while exotic birds were generally the least observed and were sometimes omitted from analyses due to small sample sizes.

Such large differences in sample size may lead to erroneous conclusions from dietary analyses that rare birds visit few plant species. Loiselle et al. (2007) used rarefaction analysis to compare dietary breadth of two species of manakins (Pipridae) to account for differences in sampling size of the two species. Rarefaction allows us to estimate the breadth of data we would expect to collect if we had a smaller sample, thus providing comparison between small and large samples, i.e. a correction for sampling effort. Rarefaction is useful in the instance of dietary data because we might expect that estimated breadth of diet is related to the sample size of individuals.

Burns (2012) applied this effort-correction analysis to bird diet and found observed dietary diversity was positively related to the number of observations of a given avian species. This included a re-analysis of the O'Donnell and Dilks (1994) data. In a fenced reserve near Wellington (Burns, 2012) and the re-analysed Westland data, blackbirds consumed a range of fruits similar or wider than native birds. Both sites are dominated by native plants so neither study assessed composition of native and exotic fruits in the diet.

Therefore, we have reason to suspect differences in sample size will affect the conclusions drawn from diet studies. Could the methods of previous frugivory studies skew their conclusions? Walking transects (e.g. O'Donnell and Dilks, 1994) may reduce the apparent importance of the quieter or more easily disturbed introduced birds. While kereru are generally quiet when they are stationary, they are still a large target to spot and can be especially noisy when moving about. Bellbirds and tui tend to be raucous and unconcerned by human presence (or even attracted to it) so are also easy to spot, and silvereyes can also be loud and may move about in small flocks, increasing observability. Blackbirds and song thrushes, however, tend to be inconspicuous within forest environments and react negatively to the approach of observers. During my time working in the Port Hills sites, I rarely observed either species outside of explicit focal tree observations. Although I often heard one or the other species, frequently the only sight I saw was a bird alarm calling as it flew away from me. A similar effect may have been operating in the O'Donnell and Dilks (1994) study, in which 72.1% of blackbird feeding observations had an unidentified food type (cf. tui 35.1%, kereru 15.5%, bellbirds and silvereyes both 0%) suggesting that these birds were unable to be observed even though their presence was noted. This possible bias carries over into the Kelly et al. (2006) review, as 24 of their 39 cases came from

O'Donnell and Dilks (1994).

Mist-netting birds may also bias sample sizes. Mist-netting is known to bias toward certain bird species, with the hardest birds to catch being those that are territorial, with short or infrequent flights through the forest (Remsen and Good, 1996). Additionally, if species use different tiers of the forest this can affect capture (e.g. Fitzgerald et al., 1989). This effect has also been observed in bats, where different species and guilds utilise different forest strata and setting low mist nets is known to bias toward frugivorous species (Bernard, 2001; Galindo-Gonzalez and Sosa, 2003). Because of these potential inter-specific differences in capture efficiencies, several authors have cautioned against the use of mist nets to estimate relative abundance of different species (Jenni and Leuenberger, 1996; Silkey et al., 1999). In New Zealand forests, song thrushes seem to be difficult to mist-net because they do not tend to fly in long bursts through the forest, reducing the likelihood of being caught in nets (Nicola Congdon, pers. comm.). Williams and Karl's (1996) data may reflect this, with blackbirds, song thrushes and starlings combined making up 13% of total birds caught (although we do not know how these catch rates compare to actual abundances, which are unknown). Mist-net data may also bias against some plant species; for instance, if there is a tendency for some seeds to be spat out or regurgitated (e.g. larger seeded species, McConkey et al., 2004), these may not be collected during mist-netting. Regurgitation can still result in seed dispersal particularly in birds that spend little time in the trees they feed in (thus already having moved away prior to regurgitation). However, for unknown reasons regurgitation of seeds, while common overseas, is extremely rare in New Zealand frugivory systems (Wotton et al., 2008).

I collated information on fruits of exotic and native plants in the diets of endemic and non-native frugivorous birds, largely from information provided in Higgins et al. volumes 3 (1996), 5 (2001) and 7 (2006), supplemented with data from O'Donnell and Dilks (1994), Williams and Karl (1996) and Kelly et al. (2010) (Table 4.9). In total, I listed 182 plant species with fleshy fruits consumed by birds; 104 (57%) native species and 78 (43%) exotic species. I only collated data for seven bird species I knew to include a large amount of fruit in their diet and which are widespread in New Zealand; bellbird, blackbird, kereru, silvereye, song thrush, starling and tui. All species I looked at, except starlings, are recorded feeding on more native plants than exotic plants. There were more records for native bird species.

Questionnaires of ornithologists in the United States of America reported avian consumption of fruits of 17 non-native plant species that had not been assessed for potential invasiveness in the respective areas (Aslan and Rejmánek, 2010). Three of these plant species were then studied in detail and confirmed that the questionnaire results were accurate in identifying these species

Table 4.9: Records of native and non-native birds feeding on fleshy fruits of native and exotic plants. Collated from: O'Donnell and Dilks, 1994; Higgins and Davies, 1996; Williams and Karl, 1996; Higgins et al., 2001, 2006; Kelly et al., 2010.

Species	Native plants (%)	Exotic plants (%)	Total
Native			
Bellbird	57 (81)	13 (19)	70
Kereru	69 (76)	22 (24)	91
Silvereye	55 (59)	38 (41)	93
Tui	52 (81)	12 (19)	64
Total	**93 (73)**	**35 (27)**	**128**
Non-native			
Blackbird	40 (62)	25 (38)	65
Song thrush	19 (70)	8 (30)	27
Starling	14 (38)	23 (62)	37
Total	**44 (51)**	**42 (49)**	**86**

interactions (Aslan, 2011). Of the 182 plant species in Table 4.9, many have not been covered in prior studies of avian frugivory, thus more in depth study may validate these feeding records. Such a list of feeding observations does not account for frequency or importance in diet, however it does indicate that exotic birds are able to feed on a range of native plant species. This is congruent with the theory of "diffuse" mutualisms, discussed in Chapter 1, in which naïve animals are able to feed on a range of novel food sources.

Aslan (2011) distinguished two types of frugivores in northern California, USA; "pulse feeders" (*Sturnus vulgaris* and *Turdus migratorius*) moved across the landscape in flocks, visiting stands for several days at a time and consuming most of the fruits available, while "background feeders" were constant in low levels in stands throughout the fruiting season. Starlings exhibit the same kind of flocking behaviour in New Zealand. In West Melton (Christchurch), birds fed in small flocks (up to 30 birds) during the breeding season, and up to 150 birds over the rest of the year (Coleman, 1977). Because in some locations starling flocks frequently fly to offshore islands at dusk to roost (Heather and Robertson, 1996), the possibility of starling flocks dispersing seeds to these islands has been studied both near Wellington (Ferguson and Drake, 1999) and near Auckland (Anderson et al., 2006). Ferguson and Drake (1999) found 66.9% of the seed

rain on Mana Island was from native woody species; these seeds are presumed to be dispersed largely by starlings since blackbirds and silvereyes both inhabit Mana island in low densities. I observed starlings on only one day during my bird observations, and on that day there were several starlings observed. This fits with the flocking behaviour and pulse feeding described above. Unfortunately, I could not observe at the same site in the days following the starling observations due to inclement weather; had I been able to continue observations the following day, it could have been interesting to see whether the starlings stayed in the site feeding for several days as seen by Aslan (2011).

In comparison, all the other small bird species I observed are likely to be background feeders; bellbirds, blackbirds and song thrushes appear to keep small territories and thus probably disperse seeds around small areas as the move about the forest fragments to feed. Meanwhile, as described in Chapter 3, kereru may have followed food availability, arriving in Port Hills fragments at the beginning of the autumn fruiting season.

I found in my study sites that all aggressive interactions between birds were either between two species of the same status (native/exotic) or were natives behaving aggressively towards exotics. Interspecific competition between avian frugivores at fruiting trees reduces the length of foraging bouts of subordinate species and changes their behaviour to feed at different times of day (Daily and Ehrlich, 1994). Such behaviour has been found in Tonga where flying fox residence times in trees were increased as density decreased (McConkey and Drake, 2006). Tui have been found to be dominant honeyeaters on offshore islands where they coexist with other honeyeaters (bellbird, stitchbird). The absence of tui on Banks Peninsula could increase residence times of smaller birds (e.g. bellbirds, blackbirds) through reduced competitive interactions.

Kereru have also been found to defend fruiting trees. Clout and Hay (1989) describe a single bird defending a fruiting miro tree for four months, and estimate this bird consumed some 10,000 fruits (85% of the fruit crop). Additionally, in Pelorus Bridge although miro fruit was available, kereru often defended trees and birds denied local access moved elsewhere to feed on miro (Clout et al., 1991). Of the aggressive interactions observed during my study, 54% (15) of them were instigated by a kereru. There was no evidence that individual birds were able to dominate a tree, but the food resource was obviously a point of contention between birds. Although kereru have been observed making long distance seasonal movements (Clout et al., 1986, 1991; Spurr et al., 1992; Robertson and Hackwell, 1995) when food is abundant in an area their behaviour can be largely sedentary. Wotton (2007) radio-tracked kereru and found birds could remain in a single tree for up to five hours. Gut retention in kereru can be up to three hours for large seeds (Clout and Tilley, 1992; Wotton, 2007) which should generally ensure seeds are moved away from

the parent tree, except in those instances where a bird spends a particularly long time in a particular tree or defends that tree (e.g. Clout and Hay, 1989).

As discussed above, mist-nets may bias toward certain species and this over-representation of certain species may skew dietary data. In addition, there are several reasons to consider whether mist-netting birds is an accurate and cost-effective method of estimating bird diet. First, there is a higher monetary and labour cost associated with mist-netting; nets must be set up and taken down each day, and monitored constantly to avoid caught birds injuring themselves. There are also ethical considerations; netting birds presents a stressful situation for caught birds and one that can cause injury. While we may argue necessity for the purposes of identification banding or measurements that require handling (e.g. semen collection, immunology testing), gathering diet data may not warrant such invasive methods. Furthermore, mist-netting is indiscriminate in its capture of birds; in the case of frugivory studies, by-catch of non-target species (e.g. insectivores) provides no further input into the study. For instance, Williams and Karl (1996) caught a total of 602 birds, 138 (22.9%) of which were non-target species (e.g. sparrows, chaffinches and kingfishers). Given the labour intensiveness of this type of study, and the implications for caught birds, such by-catch is wasteful and perhaps unethical.

In contrast, few frugivory studies in New Zealand have involved focal observations on fruiting plants as I have conducted. I see no reason to entirely skip this non-invasive method by moving directly to mist-netting of birds. As I have shown, not all bird species are equal in their observability in native canopies, and short observation periods (e.g. gathered through walking transect or opportunistic sightings) may under-estimate the visitation of less abundant and quieter birds. Additionally, observing behaviour at the fruiting plant allows for inclusion of interactions such as competition and assessment of residence times, both of which affect movement of seed away from parent tree and thus provide important information for seed dispersal studies. Bird diets may reflect an actual preference, the relative availability of different food types, vertical stratification within the forest, or competitive exclusion from certain high-quality resources. New Zealand honeyeaters are known to compete for nectar resources, and these interactions have been studied in relation to competition and resource partitioning based on social structures (Craig et al., 1981; Craig, 1985; Rasch and Craig, 1988) but this aspect appears to have been left out of frugivory studies. Direct observations may be substituted with motion-sensitive cameras (still or video) positioned with a view of a fruiting plant (e.g. Prasad et al., 2009); such footage may be particularly useful in capturing noctural or unexpected visitors (e.g. Pattemore and Wilcove, 2012). Whichever method proves most useful for further studies in New Zealand frugivory, I advocate for an emphasis on focal tree observations to improve our understanding

of relative roles of frugivorous species, both native and exotic, in providing dispersal service to fleshy-fruited native plants.

Chapter 5

Seed dispersal of matai (*Prumnopitys taxifolia*) by feral pigs (*Sus scrofa*)

Matai seedlings germinating after consumption by feral pigs.

O'Connor, S-J & Kelly, D. (2012) Seed dispersal of matai (*Prumnopitys taxifolia*) by feral pigs (*Sus scrofa*). New Zealand Journal of Ecology 36(2): 228–231.

5.1 Abstract

Introduced feral pigs (*Sus scrofa)* include native fruit and seed in their diet, and thus may act as seed dispersers if seeds are passed intact. The aim of this study was to determine whether pigs consume, and subsequently disperse, intact seeds of the New Zealand native tree matai (*Prumnopitys taxifolia*). Two captive pigs were fed 100 ripe fruit of matai and their faeces checked for seeds for 4 days. Fourteen intact seeds (14%) were recovered and 57% of these germinated under glasshouse conditions, comparable to germination from hand-cleaned seeds. We collected 3.5 kg of pig faeces from matai-dominated forest in Isolated Hill reserve, southern Marlborough. This sample contained over 450 intact matai seeds; these seeds readily germinated in the glasshouse, reaching 68% germination after 22 months. These results indicate that pigs are consuming native fruit and passing some viable seeds out - thus acting as occasional seed dispersers.

5.2 Introduction

Feral pigs (*Sus scrofa*) include fleshy fruits and seeds as part of their diet both in New Zealand and elsewhere (Henry and Conley, 1972; Wood and Roark, 1980; Thomson and Challies, 1988). However, pigs are known to grind up seeds in the process of consumption (Beveridge, 1964; Fedriani and Delibes, 2009) so this behaviour has rarely been linked to legitimate seed dispersal. Mammals have the potential to be important for long-distance seed dispersal because of their long gut passage times and wide-ranging behaviour (Jordano et al., 2007) and have been found elsewhere to disperse seeds (Abe, 2007; Calvino-Cancela, 2011; Shiels and Drake, 2011). Here we test whether feral pigs are potential seed dispersers of a medium-sized native seed (matai, *Prumnopitys taxifolia*, mean fruit and seed diameters 9 and 7 mm respectively). Matai is a gymnosperm, so technically produces single-seeded cones, but for simplicity we will use the term "fruits" for all kinds of fleshy-coated diaspores.

Feral pigs occupy c. 35% of New Zealand, including 13 islands (Fraser et al., 2000). In theory, if pigs disperse a wide variety of native seeds, this could partly compensate for the decline or disappearance of native frugivores (Kelly et al., 2010). Northern Hemisphere diet studies show plant material makes up approximately 80% of the diet of feral pigs. Certain foods are readily consumed seasonally, especially mast seed crops such as acorns and hickory nuts (Henry and Conley, 1972; Wood and Roark, 1980; Baber and Coblentz, 1987; Massei et al., 1996; Schley and Roper, 2003).

A New Zealand study of pig diet showed plant material made up 72% of the annual diet, including two fruits (tawa *Beilschmiedia tawa* and hinau *Elaeocarpus dentatus*) that together comprised 30.9% of the diet (Thomson and Challies, 1988). Fruits of matai, supplejack (*Ripogonum scandens*), miro (*Prumnopitys ferruginea*) and nikau (*Rhopalostylis sapida*) are also eaten (Beveridge, 1964; Knowles and Beveridge, 1982; Thomson and Challies, 1988). None of these studies recorded whether any seeds were viable, although Beveridge (1964) noted that "a large quantity" of seeds were crushed.

Although fruit is common in pig diets, studies have been divided on to what extent they destroy the consumed seeds versus passing them out intact. In Hawaii, pigs disperse viable seeds of the introduced *Passiflora mollissima* (LaRosa, 1992). Work in New Zealand confirmed that pigs consume *Passiflora mollissima* fruit and excrete most of the seeds intact and viable (Beavon, 2007). In Australia two-thirds of mesquite (*Prosopis pallida*) seeds consumed by feral pigs were undamaged; approximately 80% of these seeds then germinated (Lynes and Campbell, 2000). However, in Argentina Campos and Ojeda (1997) found that all *Prosopis flexuosa* seeds found in pig faeces had been destroyed. Consequently, the presence of fruit in pig diets does not necessarily equate to seed dispersal.

We studied the viability of seeds of a native tree, matai, following consumption by pigs. We hypothesised that pigs consume this native seed and release some intact in faeces, thereby acting at least partially as seed dispersers. Our study has two parts: first we conducted a feeding experiment using captive pigs to determine whether the resulting faeces contained viable seeds; second, faecal samples were collected from a patch of matai-dominated forest to test whether intact seeds were also present in wild pig faeces.

5.3 Methods

5.3.1 Captive trial

Two adult, female kunekune pigs (*Sus scrofa*), a New Zealand breed, were kept in captivity at Willowbank Wildlife Reserve (Christchurch, New Zealand). Both were born in captivity and weighed approximately 130 kg. One hundred ripe matai fruits (one seed per fruit) were fed in one session to the two pigs, with each eating approximately 50 fruits. Most of these fruits were hand-fed to the pigs, with others sandwiched between bread (for easy handling) or placed straight into the feeding trough. All seeds were ingested, none were spat out. Fruits had been collected from the Port Hills,Christchurch, mostly under a single female matai, approximately one month earlier, and refrigerated. Only ripe fruits were used.

The entire enclosure was cleaned of pig faeces within 30 min of the seeds being ingested. Pig faeces were then collected during daily cleaning for four consecutive days. These samples were taken back to the laboratory and sifted to recover seeds.

5.3.2 Field study

Field evidence of pig dispersal was collected in winter 2008 from Isolated Hill, a 2,835-ha scenic reserve in southern Marlborough, New Zealand. Matai-dominated forest makes up 8% of the reserve (Williams, 1982). Feral pigs are present throughout the reserve, being especially abundant in areas containing matai (Cochrane, 1994). We observed on initial visits that pig faeces in this area appeared to contain large amounts of crushed matai seeds. On a single day in June 2008 a 3.5 kg sample of pig faeces was collected from under c. 12 female matai trees through a 1-ha area of matai-dominated forest within the reserve (E2591170, N5924350, NZMG). Although pig faeces were most easily collected under female matai trees, given the long gut passage times of pigs (see Results), matai seeds are likely to be present in faeces deposited elsewhere. The samples were collected 1–2 months after matai had finished fruiting; all faeces were solid and water was required to sift samples and retrieve seeds.

5.3.3 Germination

All intact seeds recovered from both the captive and field trials were potted in June 2008 and kept under glasshouse conditions as for previous studies (Kelly et al., 2010) at the University of Canterbury, Christchurch, New Zealand, for two spring/summer cycles until June 2010. Germination was recorded at least monthly, but weekly during the main period of germination (November to March each year for 2 years). We were unable to measure the germination rate of non-pig processed seeds ourselves, as all the available fresh fruits collected were used in the captive feeding trials. We therefore compared our data to hand cleaned and germinated matai seeds presented by another study (Kelly et al. 2010, their table 2). The same glasshouse was used as for Kelly et al.'s study, but their seeds were sourced from Pelorus Bridge (Nelson) and Blue Duck Reserve (Kaikoura). In addition, we potted 20 seeds from Isolated Hill that were partially damaged from pig mastication. Overall germination success rates were compared between trials using chi-square tests on the totals.

Table 5.1: Germination tests on seeds of matai (*Prumnopitys taxifolia*) that have passed through captive and feral pigs. Seeds were sown in a glasshouse (University of Canterbury, Christchurch, New Zealand) and germination recorded over two spring–summer cycles.

	Captive trial: Intact seeds	Field study: Intact seeds	Field study: Partially damaged	Hand-cleaned (Kelly et al., 2010)
No. planted	14	472	20	200
No. germinated				
Spring–summer 1	5	115	3	114
Spring–summer 2	3	208	5	13
Total germinated (n)	8	323	8	127
Total germinated (%)	**57.1**	**68.4**	**40.0**	**63.5**

5.4 Results

5.4.1 Captive trial

The captive pigs readily ate the matai fruits offered to them, consuming all within 5 min. The pigs made loud crunching sounds as they chewed the seeds. Despite this, of the 100 seeds fed to the pigs, 14 intact seeds were retrieved from the collected faeces. The seeds were excreted over 4 days, with most collected on Day 3. In total 8 of the 14 seeds (57%) germinated in the glasshouse – seven in spring 2008, one in spring 2009 (Table 5.1). As the Day 4 sample still contained some matai seed we cannot exclude that some seeds were excreted after the trial ended. The faeces contained an unquantifiable amount of shattered matai seed coat; no partially damaged seeds were recovered.

5.4.2 Field study

The 3.5 kg of pig faeces collected from Isolated Hill contained abundant matai seed-coat fragments (not quantified), many intact matai seeds (c. 470), and some damaged seeds; 20 partially damaged matai seeds (potted in glasshouse) and 20 matai seeds broken in half with traces of the embryo present (not potted). If we apply the 14% recovery rate of intact seeds from the captive trials, then this would suggest approximately 3,400 fruits were consumed by the pigs in question. No other food sources were identified in the faecal samples, indicating pigs were concentrating

on matai fruit in the area sampled at that time. There were no intact seeds from other plant species.

5.4.3 Germination

Glasshouse seeds began germinating in November 2008, 6 months after being planted. Germination continued throughout the next year, with low but consistent numbers over winter, followed by a second flush beginning in September 2009. No further germination was recorded after March 2010 (22 months after the seeds were planted). All three samples (hand-cleaned, captive, field) reached approximately 60% germination. We compared total germination success between the hand-cleaned seeds (from Kelly et al. 2010), captive trial and field trial. There was no significant difference between hand-cleaned seeds and either captive trial data (χ^2 = 0.04, df = 1, p = 0.85) or field data (χ^2 = 1.33, df = 1, p = 0.25). An additional 8 seedlings germinated from partially damaged seeds obtained from feral pigs faeces.

5.5 Discussion

This study has shown that feral pigs consume matai fruits in the wild and deposit a proportion of intact, viable seeds following gut passage. Though pigs are known to disperse introduced weeds (Beavon, 2007), this appears to be the first New Zealand report of viable native seeds being dispersed by pigs.

Generally, pigs have been thought to be largely nomadic (Wodzicki, 1950); however, studies both in New Zealand and elsewhere have shown distinct home ranges and small daily travel distances (Martin, 1975; McIlroy, 1989; Saunders and Kay, 1991; Caley, 1997). New Zealand studies showed home ranges up to 2 km^2 and immature pigs moving up to 3 km in a 24-hour period (Martin, 1975; McIlroy, 1989). Mammalian home ranges can be much larger than those of birds (Jordano et al., 2007).

This slow gut passage of seeds (2–4 days) has large implications for potential dispersal. With pigs possibly covering several kilometres in a 24-hour period (McIlroy, 1989), seeds could be moved kilometres away from the parent tree. Also significant is that seeds were defecated over the space of 3 days. Rather than all seeds consumed at the same time being defecated in one spot, they could be spread over a larger area, reducing spatial clumping of seeds. Long gut passage times may negatively affect seed germination, as has been found in other studies (Fedriani and Delibes, 2009), but no such lowered germination was found in matai when compared with hand-cleaned seeds. This may be due to the thick endocarp of matai (Thorsen et al., 2009).

It is possible that other introduced mammals may provide some dispersal services to native plants, as demonstrated with ship rats (*Rattus rattus*) in Hawaii (Shiels and Drake, 2011) and Japan (Abe, 2007), rabbits (*Oryctolagus cuniculus*) in Australia (Calvino-Cancela, 2011) and carnivores (badgers, foxes and stone martens) in Spain (Jordano et al., 2007). In New Zealand, ship rats passed only very small seeds intact (Williams et al., 2000), while brushtail possums (*Trichosurus vulpecula*) have been found to include small fruit in their diet, and excrete intact and viable seeds (Dungan et al., 2002). Matai seeds have been retrieved from possum faeces and germination percentages of 40–50% recorded (J. Ladley, University of Canterbury, pers. comm.). However, the importance of possums as dispersers of larger native fruit is still controversial (Williams, 2003). There is little information about possible seed dispersal by larger introduced mammals such as deer or feral cattle (Kelly et al., 2010).

While pigs may make a relatively small numerical contribution to dispersal of native seeds, in New Zealand's modified environment this may assist in maintaining dispersal. The loss of native avifauna has been well documented (Bell, 1991), and the implications for mutualisms discussed and studied (Clout and Hay, 1989; Kelly et al., 2004, 2010). In several cases it appears that, despite reduced densities of native birds and little contribution by introduced birds to fruit dispersal (Kelly et al., 2006), adequate dispersal service is still being provided to native plants (Kelly et al., 2010; Wotton and Kelly, 2011). However, pigs could provide an important addition to the current suite of dispersers, given their likely long-distance dispersal. This is similar to the study of Jordano et al. (2007) which showed that c. 85% of dispersed *Prunus mahaleb* seeds in Spain were moved by frugivorous birds, but that terrestrial mammals (badgers, foxes and stone martens) contributed 67% of long-distance dispersal events.

This study has shown that feral pigs are consuming and passing intact viable seeds of one New Zealand species. Coupled with daily movement across several square kilometres, feral pigs are probably important in the long-distance dispersal of seeds, both of native plants and of introduced weeds.

Chapter 6

Synthesis

A remnant, malformed matai growing in a paddock, Prices Valley.

6.1 Dispersal of matai on the Port Hills

The aim of this thesis was to examine how dispersal of matai functions under the current conditions of fragmentation and an altered disperser assemblage. Although I was unable to develop microsatellites to the point where they could be used to measure dispersal, in particular to examine whether seeds were moving between nearby fragments, I did discover some aspects of matai dispersal. In Chapter 3 I showed that a large percentage of seeds caught under female canopies had been processed by a bird, and in Chapter 4 I found that there were five avian species responsible for this dispersal.

Matai has an intermediate-sized fruit (c. 9 mm diameter), and I found that in fragmented habitats a range of native and non-native birds fed on these fruits. However, matai fruits are still mostly too large for silvereyes to swallow readily, as I found through the scarcity of silvereye feeding observations. Kelly et al. (2010) do not list silvereyes as feeding on matai, and my observation was likely a rare event; Ladley and Kelly (1996) observed silvereyes "whacking" mistletoe fruits down their throats, so we know these birds can swallow larger fruits on occasion. Additionally, Kelly et al. (2010) report bellbirds as having a mean gape smaller than the mean diameter of matai, however bellbirds contributed 25% of observed fruit-feeding visits to matai.

Our knowledge of dispersal service to a range of fruiting species would benefit by conducting focal tree observations on additional species; for instance a smaller fruited species (i.e. $<$ 6 mm) and a larger fruited species (i.e. $>$ 10 mm). Along with matai, we could build a framework for assessing dispersal service by studying frugivore visitation to these focal species across a range of habitats. Thus, we can study dispersal within fragmented environments, as I have done here, and compare that to service in intact forest. Additionally, we can compare service with different assemblages of dispersers, e.g. with and without tui (Canterbury and elsewhere), with and without bellbirds (Northland and elsewhere), and with and without rarer birds (e.g. saddleback, stitchbird, kokako). There are many opportunities to untangle the complex relationships of diffuse dispersal mutualisms, and by including a range of fruit sizes and a range of disperser assemblages we may be able to elucidate which factors drive dispersal service and better understand how mutualisms have been affected by the recent history of extinctions and introductions of dispersing species.

Introduced mammals are increasingly being considered as potential dispersers of native and exotic seeds. In this thesis I have confirmed that feral pigs (*Sus scrofa*) consume and disperse intact seeds of matai. This research was based on observations my supervisor, Dave Kelly, had made in Isolated Hill some years prior of what appeared to be pig faeces packed full of cracked

matai seeds. Through field collection followed by a germination experiment, I was able to confirm these observations. As noted by Aslan (2011), observations of the presence of foods in animals diets can be used as a starting base for further study; thus, we could expand our knowledge of mammal dispersal of native fruits by beginning with those species for which we already have some indication that frugivory occurs, e.g. *Beilschmedia tawa* and *Elaeocarpus dentatus* for pigs (Thomson and Challies, 1988). By beginning with species combinations we already have diet evidence for, we will hopefully gather the most amount of data per expended effort. The longest period in such research will be the time lag waiting for germination to occur, but this is essential evidence in deciding whether a given mammal adversely affects germination and cannot be skipped. My study presented in Chapter 5 would have been elevated in its strength had I been able to assess the viability of ungerminated seeds, but logistical difficulties prevented this. Future studies should build this into their experiments in order to provide the strongest results; particularly for species such as matai which take a long time to germinate, and often do not germinate to 100%, it would be beneficial to know whether remaining seeds were dead, destroyed or dormant.

Previous authors have commented that podocarp regeneration is abundant in certain Port Hills sites (e.g. Wilson, 1994); to this I can add my own observations that in Ahuriri Summit, where fencing is effective at excluding large herbivores, matai regeneration is abundant. Although I did not quantitatively measure seedling and sapling distribution, my observations indicate that seedlings are abundant and the presence of several small poles suggests there has been recent recruitment. Ahuriri Summit is the best fenced of the sites I studied; there are no large ungulates, some possums (with control underway) and I observed occasional rabbits. This would suggest the browsing pressure in this site is low. Ahuriri Summit is also the only site where I observed kahikatea and totara seedlings. In Kennedys Bush, recent regeneration was not as prominent, however there is an abundance of saplings perhaps suggesting a period of regeneration that has ended. For instance, Figure 6.1 shows a dense patch of matai saplings growing in a canopy gap in Kennedys Bush. There are two adult female matai and an adult totara growing nearby, but these saplings have been dispersed to a rare canopy gap and seem to be thriving. Although Kennedys Bush is well fenced, I observed goats within the forest on one occasion, perhaps suggesting a weakness in the fencing regime and a possible explanation for the perceived lack of recent regeneration. Overall, there is evidence that even in these highly modified and fragmented forests on the Port Hills, dispersal service is being provided by a suite of frugivores. Thus, regeneration is continuing in sites where herbivory pressure is low.

Figure 6.1: Matai saplings (c. 12 stems) growing in a thick clump in Kennedys Bush. A adult female matai is visible in the background, an adult totara and another female matai are out of shot but also grow close to these saplings.

6.2 Further use of microsatellites

Through my Ph.D. research I have developed a new study system on which we can now base future research on seed dispersal, pollen dispersal and the effects of fragmentation. Setting up the new system has been labour intensive, as I began work on a species not previously used by our research group, mapped and sexed adult trees at a range of sites across the Port Hills, and developed expensive and time-consuming microsatellite primers. Now that this baseline work has been done, there are several promising avenues this research could now take.

Across two of the Port Hills sites (Ahuriri Valley, Tai Tapu) I collected pig faeces containing matai seeds; we now know that feral pigs are legitimate, albeit occasional, dispersers of matai (Chapter 5). With that confirmed, any seeds collected from pig faeces do not need to be saved for germination trials, and instead can be dissected for DNA extraction and genotyping at microsatellite loci. Such work will allow for a separate dispersal kernel to be calculated from pig dispersed seeds, as opposed to bird dispersed, allowing analysis of the dispersal behaviour of different components of the disperser assemblage (e.g. Jordano et al., 2007). Large mammals

have been found to have much longer gut passage times (e.g. pigs 2–4 days, Chapter 5) and range across much larger areas than birds, and therefore pigs may be an important provider of occasional long distance dispersal events. With pigs present in at least two sites within the Port Hills study area during this study, such collection of faeces and analysis of seeds provide an easy way to quantify uncommon events. Rare long distance dispersal events are likely to be important in overall dispersal, and infrequent dispersal agents such as pigs and introduced starlings may prove important in providing such events in the current highly modified environment.

Currently we know almost nothing regarding pollen dispersal in the New Zealand conifers. Although there have been anatomical and morphological studies into the structure of podocarp male cones (Tomlinson, 1992, 1994), we do not know how far pollen is moved between male trees and their final destination on female cones. Although, at least for matai, there is reason to suspect distances may be great; matai pollen turns up in pollen cores in areas adult trees probably did not grow (e.g. alpine peats) (McGlone, 2001). Should wind pollination of New Zealand Podocarpaceae be on the same scale as that seen in Northern hemisphere conifers, it is possible that even highly fragmented populations of conifers will be able to pass pollen between them, thereby acting as a panmictic population (Ashley, 2010). Such large scale movement of pollen may maintain gene flow despite the highly fragmented nature of New Zealand forests. In fact, by studying across a landscape such as my study system with multiple fragments in close proximity, we might find that wind pollination is less affected (or even improved) by fragmentation than animal-mediated seed dispersal.

There are two methods by which pollen dispersal may be studied using microsatellites. The first follows the methods conducted by the majority of molecular studies in pollen movement. Seeds are collected from the canopies of seed parents and their embryos genotyped. As the seed parent is known, the pollen donor can be inferred via exclusion probabilities and the distance between the pollen donor and seed parent used as the measure of seed dispersal distance. But as I mentioned in Chapter 2, gymnosperms have the peculiarity of a haploid megagametophyte, and this has been used to aid in the inference of paternity. Using this method, it is not necessary to take seeds directly from the seed parent; instead, seeds can be collected across the landscape (as for a seed dispersal study) and both parents inferred from the respective genotypes of the megagametophyte and the embryo (Iwaizumi et al., 2007, 2010). Therefore, from a single seed it is possible to measure both the pollen dispersal distance and the seed dispersal distance, something which is still difficult to measure and therefore rarely have both been measured in a single species. In studies where both parents have been analysed, these have generally used seedling genotypes which (due to meiotic segregation) complicates parental exclusion (Hardesty et al.,

2006; García et al., 2007).

An additional benefit to further genetic studies of matai is that due to the long life span and slow growth to reproductive age the reproductive population will not change drastically in the space of a few years. That is, growth of new adults will not confound analysis of old results (Herrera, 2009; Garcia et al., 2009).

Along with the greater information toward solving dispersal paradoxes (see Chapter 2 introduction), molecular analysis of pollen and seed dispersal appears to be challenging Daniel Janzen's "living dead" hypothesis (Janzen, 1986). Janzen argued that remnant trees, such as those standing in isolation amongst pasture land, contribute nothing to the reproduction of the species and are therefore of little conservation value. However, if pollen and seed are able to move such large distances as are being measured via molecular methods, these remnant trees may still be able to exchange genes with nearby fragments (Ashley, 2010). Remnant trees have been found to provide important habitat and food resources for frugivores and may enhance seed dispersal and colonisation of matrices by attracting frugivores (Guevara et al., 1986; Guevara and Laborde, 1993; Aldrich and Hamrick, 1998; Manning et al., 2006; Gibbons et al., 2008; Manning et al., 2009). Such remnant matai trees are common across Banks Peninsula (see frontispiece, this chapter) and could retain conservation value through the movement of genes. Finding a sample of these remnants and including them in genetic analyses, such as that I have described above, could provide further information for or against Janzen's living dead hypothesis and support New Zealand's conservation views which tend toward maintaining such remnants.

If species are closely related, species-specific microsatellite primers may be able to be used across species. The obvious candidate for such cross-study in this case would be miro, *Prumnopitys ferruginea*, the other New Zealand member of *Prumnopitys*. As I mentioned in Chapter 1, the phylogeny of Podocarpaceae has been questioned, in particular the grouping of *Prumnopitys*. Matai and miro, though currently grouped into the same genus, may with further study be moved into distinct genera. Should the two species be as closely related as current taxonomy suggests, there is a possibility that the microsatellite primers I have developed will cross-amplify in miro. This would be an interesting experiment, and one that may shed further light on the relationship of the two species. Now that we have some genetic information on matai, even if the primers do not amplify (or perhaps particularly if they do not) there may be interest in sequencing sections of miro's genome to search for interspecific differences.

6.3 Research on private land

In setting up a new study system based around fragmentation, private land will likely be an option to work in if the goal is to cover all possible fragments in a landscape. In the course of this study, I have worked across a range of conservation levels, from highly protected with fences and formal pest control to fragments largely unmanaged. This variety of field sites has thrown up interesting observations on both the challenges of working in non-protected areas and the potential conservation values of privately owned land.

Unprotected sites, whether privately owned or not, are largely unsuitable for certain research; for instance, in this study I struggled to use ground-based seed traps in forests with frequent introduced ungulates because the ungulates disturbed and damaged (i.e. kicked!) traps. However, certain research, such as herbivore exclusion, can only be conducted in these sites with their large herbivores. Additionally, as I have shown, some of these large animals may be legitimate dispersers (Chapter 5) and by proxy, gathering information on such behaviour requires "non-pristine" areas. In fact, one of the sites that was largely unusable for seed trapping (Tai Tapu) had a frequent ungulate presence due to ineffectual fences, and it was in this site that I was able to collect pig faeces containing matai seeds as I did at Isolated Hill for Chapter 5. I saved these Tai Tapu seeds in the hope of using them for genetic analyses (Chapter 2), which would have allowed explicit comparisons of bird dispersed (Chapter 3) and mammal dispersed seeds. If I only worked in intact forest with good fences and pest control, such non-standard dispersal agents would not be present. I also found the importance of field observation in deciding whether fencing regimes were effective, as large herbivores were sometimes seen in sites that were meant to be fenced and ungulate free (Figure 6.2).

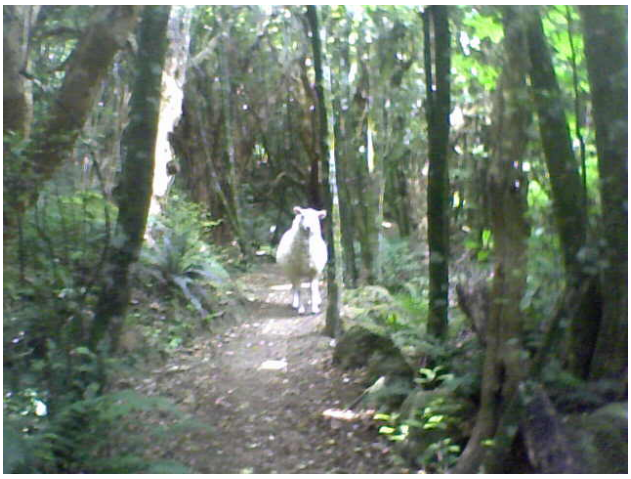


Figure 6.2: A stray sheep in Omahu Bush, a supposedly "fenced" site.

Globally, New Zealand has one of the highest proportions of land area under conservation protection, yet the bulk of this land is high altitude and steep (Norton, 2000). Because lowland ecosystems hold high economic value, these are the ecosystems that bear the largest burden of human activity. As I described in Chapter 1, Banks Peninsula experienced rapid deforestation after European arrival and native habitat across the peninsula now exists largely as isolated fragments permeated by invasive species.

Norton (2000) emphasised that despite acknowledging that private land deserves protection, we are still limited in our understanding of the ecological processes operating in these highly modified environments. What research there is has been focused on remnants rather than landscapes (Norton, 2000). Through my study system, I have provided an example by which we can study both remnant and landscape processes, incorporating a range of property types. As David Norton (2000) points out, "working with remnant species in muddy fields among cows" is not as glamorous as working with rare and iconic species in remote national parks, neither can sidestepping cow pats to reach a remnant forest fragment be considered exotic field work. And yet these fragments are a crow's flight from a metropolitan city; they ought to be studied and understood, as pieces of history and as the closest to natural forest many city dwellers have access to.

6.4 Confronting xenophobia

> "Ultimately, non-native species are just species."
> - Mark Davis, (2011)

A running theme in this thesis has been the potential for non-native animals to provide positive services to native plants. Along with finding that feral pigs function as seed dispersers of a native plant, I have also found three non-native bird species contributing to the dispersal of matai.

It has been suggested that biases persist against non-native species, which is particularly evident in the often militarised or xenophobic language used to discuss such species (e.g. "war on aliens", cited in Schlaepfer et al., 2011). Such a persistent bias is concerning if there is a resultant publication bias against studies that fail to find a negative effect (Schlaepfer et al., 2011). Daniel Simberloff (2003) argues that such "xenophobia" is not grounded in fact, and that attitudes towards introduced species are focused on those which cause environmental or economic harm. The last few years have signalled a shift in our way of considering non-native species, with some authors advocating a re-examination of the effects of non-native species (Goodenough, 2010; Davis, 2011; Davis et al., 2011; Schlaepfer et al., 2011) although there are opposing arguments

from researchers who argue that the negative effects of biotic invasions far outweigh any potential benefits (Edelaar and Tella, 2012; Russell, 2012).

Non-native species, by their nature, are those which are most likely to be tolerant and adaptable to new conditions; therefore, it is possibly these same species which will function best in the changing environments (Schlaepfer et al., 2011). Schlaepfer et al. (2011) question why we treat human-assisted dispersal as distinct from natural vicariant dispersal of species into new ranges (dispersal ecologists in particular ought to be open to the possibility of expanding species ranges, Westcott and Fletcher, 2011). We in New Zealand are guilty of calling this distinction; where blackbirds and the like are "introduced" birds, silvereyes are often described as "naturalised", "self-introduced" or even "native" (Heather and Robertson, 1996). Such a distinction suggests a demarcation between the two groups, and while we have no evidence for deliberate introduction of silvereyes to New Zealand, they were not present prior to European arrival (the Maori name for silvereye, tauhou, roughly translates to "stranger" giving us an indication that they had not been observed by early Maori). Silvereyes from Tasmania, Australia, first colonised New Zealand's South Island in 1830; by 1956 birds had arrived in Chatham Island and the North Island (Palmerston North); by 1865 birds had colonised Auckland and in 1904 these bird from the upper North Island had arrived in Norfolk Island (Clegg et al., 2002). It would be quite a coincidence if silvereyes arrived in New Zealand at the same time as Europeans completely without connection; more likely, their arrival was facilitated by European arrival, perhaps by ships passing Australia on their way to New Zealand or else birds had been arriving earlier but required the land transformation that occurred when Europeans arrived to facilitate colonisation. Once arrived, silvereyes spread rapidly across the country. And yet this simple distinction, between deliberate introduction and possible facilitated dispersal, is enough to change the way we treat avian species. If we were to take the advice of Schlaepfer et al. (2011) and use the terms long-term resident, recently arrived, and new species, we will still be able to add our own category of "endemic" species which are important to us socially and ecologically, while perhaps shifting our current paradigm to consider the possibility of non-native species having neutral or positive effects in their new environment.

Unexpected animals may function as seed dispersers in New Zealand. I have shown feral pigs to be legitimate, if occasional, dispersers of matai (Chapter 5), while the New Zealand falcon (*Falco novaseelandiae*), typically assumed to be a secondary seed disperser, has been observed conducting primary seed dispersal of native alpine plants (Young and Bell, 2010), and kea (*Nestor notabilis*), presumed like most parrots to be primarily seed predators, have been found to be the most effective seed disperser of the alpine fleshy-fruited flora (Young et al., 2012). These exam-

ples illustrate that assumptions of the behaviour of both native and non-native species should be treated with caution, and good scientific inquiry should delight in the ability to examine such assumptions.

There is no doubt that invasive species can wreak havoc in new environments; we know that all too well in New Zealand. But tarring every species with the same brush is a coarse way to approach a complex ecological situation. We certainly now face "permanently invaded" systems (Carroll, 2011) where eradication of many non-native species from all of the mainland is probably impossible. Recently, ship rats (*Rattus rattus*) have been found to provide some pollination service to native plants on mainland New Zealand, in the absence of the endemic birds and bats that provided service on an offshore, predator free island (Pattemore and Wilcove, 2012). However, the negative effects of rodents in New Zealand are extensive and well-documented, including direct predation on birds and insects and suppression of recruitment via seed predation or herbivory (Campbell and Atkinson, 1999; Moles and Drake, 1999; Atkinson and Towns, 2001; Innes, 2001; Campbell and Atkinson, 2002; Wilson et al., 2003; Towns et al., 2006; Gibbs, 2008; Towns, 2008). We could hardly claim that a small contribution to pollination outweighs the wealth of negative effects.

Although there is some support for the deliberate release of "taxonomic substitutes", it is unlikely that such releases will be frequent. Griffiths et al. (2011) trialled the release of an exotic tortoise (*Aldabrachelys gigantea*) on a Mauritian island as a taxonomic substitute for extinct giant tortoises (*Cylindraspis* sp.). Dispersal of the large-seeded *Diospyros egrettarum* was almost non-existent prior to the project (a count of 7437 seeds found only 7 beyond a female canopy); the 19 released animals consumed large numbers of *D. egrettarum* fruits and moved seeds across the island. As promising as such results sound, there is a great difference in releasing a large, easily monitored species such as a tortoise (researchers were able to keep animals within a penned area in the early stages of the trial to assess impact) and deliberately introducing highly mobile species such as birds that would be nearly impossible to monitor and control long term. Such additional releases, for example of pollinating birds (e.g. hummingbirds) or frugivores (e.g. fruit doves) would pose risk of disease introduction and competitive exclusion of native birds. Hence, particularly in a country like New Zealand, it is unlikely taxonomic substitutions will become popular, even though the idea has been posited (Atkinson, 1988; Parker et al., 2008).

However, we already have non-native species potentially acting as some degree of taxonomic substitute (perhaps more so for dispersal than for pollination). True, blackbirds may not be as exotic and sexy as introducing bowerbirds (e.g. *Scenopoeetes dentirostris*, Atkinson, 1988), but these birds once held enough cultural meaning that early European settlers worked hard to bring

birds with them and establish them in their new homeland.

This thesis has provided additional data about the functioning of mutualisms in today's modified environments, and has shown examples of several non-native species which may have positive effects on the seed dispersal of native plants. Working in intact forest with endemic species is all well and good for figuring out what the "natural" condition is, but even then we will never return to the assemblage present before human impacts. With our current information, it appears dispersal of fleshy-fruited species is more or less functioning in today's modified environments. Non-native species, although contributing a numerically minor part of frugivory, may provide important long-distance dispersal and likely provide some buffer against the effects of further losses in the native, frugivorous avifauna.

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Appendix A

Port Hills field sites

Ahuriri Summit

Ahuriri Scenic Reserve (hereafter referred to as Ahuriri Summit) is considered the "best remnant of podocarp-hardwood forest in the Port Hills District" (Wilson, 1994) (Figure A.1). A small (10.9 ha), Christchurch City Council (CCC) site, Ahuriri Summit was gazetted in 1914 and fenced in 1959 (Grid Ref. M 36 796 267) (Burrows, 1994) (Wilson, 1994). This was once part of the larger Ahuriri Station, separated and gifted as a scenic reserve in 1914 (Ogilvie, 2009). Dozens of adult matai, totara and kahikatea represent pre-European forest, though many show scaring from fire (Kelly, 1972). This is the only remaining intact remnant of pre-European podocarp forest remaining on the Port Hills, highlighting the importance of this reserve. The presence of kahikatea, a species with high water requirement, in a dry area suggests particular water-storing capabilities of the soils in this site (Kelly, 1972). Podocarp regeneration, in particular matai, is vigorous within this site. There is a large population of matai seedlings, with representatives of many size classes of saplings and poles throughout the reserve.

Ahuriri Valley

Ahuriri Bush (hereafter referred to as Ahuriri Valley) is a privately owned, 80 ha reserve covering a large amount of the altitudinal range of the Port Hills (Grid Ref. M 36 775 248) (Wilson, 1994) (Figure A.2). This is the second largest reserve on Banks Peninsula, behind Mount Herbert Reserve (135 ha) (Conner, 1980). It is one of two remaining forest stands from the original Ahuriri Station (the other being the above mentioned Ahuriri Summit Reserve) (Ogilvie, 2009). The landowner has had the reserve protected under a QEII Covenant since 1980, with fencing completed in 1981 (Burrows, 1986). However, large ungulates including pigs and cows are present in the reserve (pers. obs.). The vegetation in this reserve has been extensively studied (Conner, 1980; Burrows, 2006). Two main vegetation types occur; large tracts of kanuka, plus areas dominated by scattered podocarps. The forest was milled during the 1850's and 1860's, following which about half

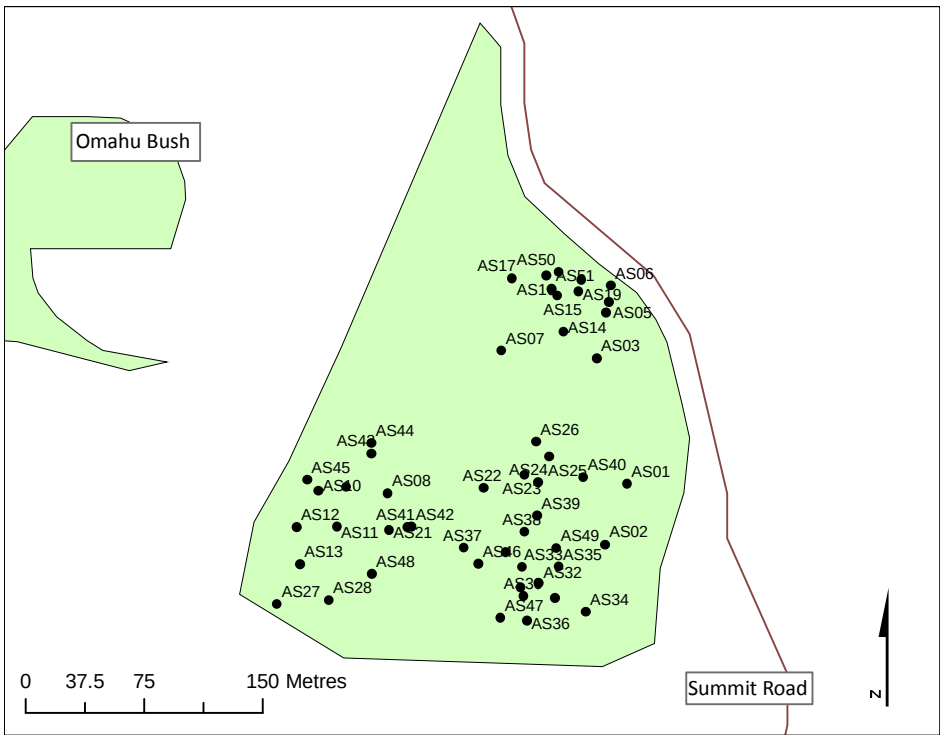

Figure A.1: Mapped adult matai trees in Ahuriri Summit.

the forest was burnt. Much of this burnt area has regenerated into kanuka canopy, with surviving podocarps showing fire scars (Conner, 1980; Burrows, 2006). These adult podocarps may represent remnants of the bush prior to European milling (Conner, 1980).

Cass Peak

Cass Peak is a small (3.2 ha) CCC reserve (Grid Ref. M 36 795 299) (Wilson, 1994) (Figure A.3). It was gazetted in 1914, and fenced in 1976 (Kelly, 1972; Loughton, 1998). Despite the small size, this reserve is notable in that over certain areas the canopy is comprised entirely of deciduous species (fuchsia and lowland ribbonwood). Kelly (1972) suggests this reflects the high natural fertility of the steep slopes Cass Peak is located on. This site does not show evidence of abundant large-ungulate damage to vegetation (pers. obs.).

Kennedys Bush

Kennedys Bush is one of the most-visited reserves on the Port Hills, and has one of the best documented histories of reserves on Banks Peninsula. It is a relatively large site (86.5 ha) with a wide altitudinal range (Table 1.1) and is controlled by the CCC (Grid Ref. M 36 795 307) (Wilson, 1994) (Figure A.3). Much of the upper slope is regenerating bush, some of which has been intentionally planted. Further down the reserve remains a handful of adult matai and totara. Harry

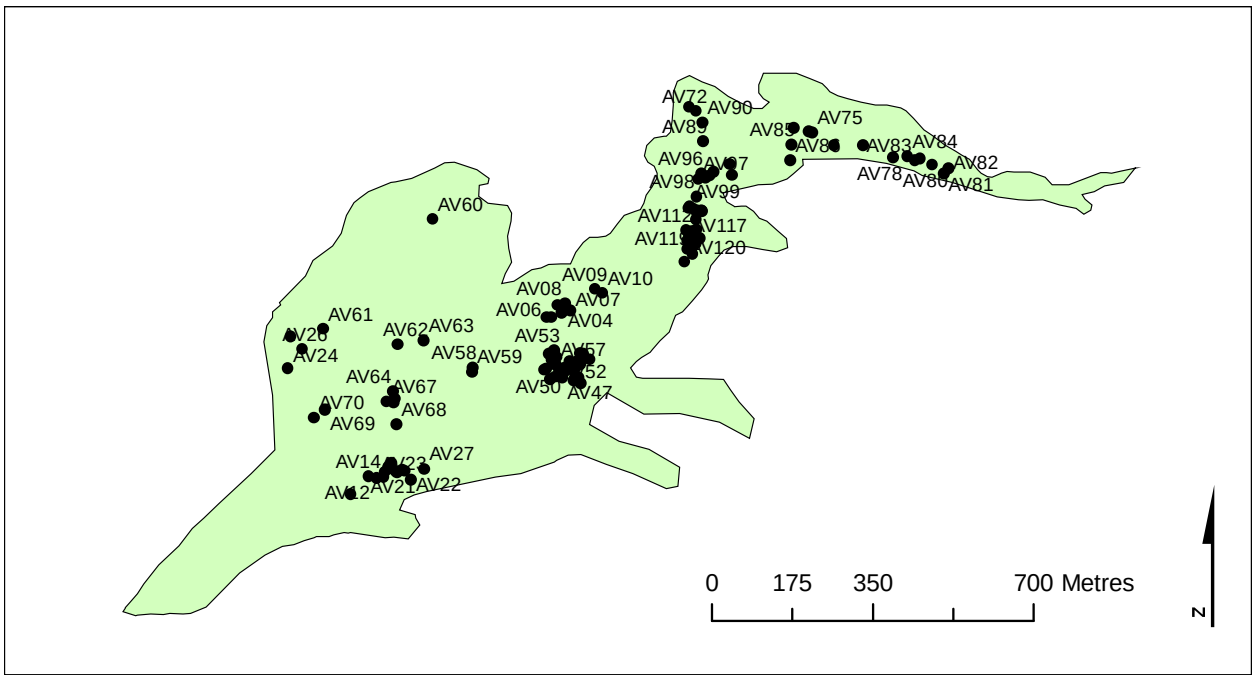

Figure A.2: Mapped adult matai trees in Ahuriri Valley. Note: not all trees have been mapped in this site.

Ell began a public campaign in 1900 to protect what remained of Kennedys Bush, and for this purpose he was the prime promoter of the Scenery Preservation Act of 1903. This allowed 21 hectares of Kennedys Bush to be gazetted in 1906, becoming the first Scenic Reserve on Banks Peninsula (Loughton, 1998). Additional land was added in 1908, however following Ell's death in 1934 bankruptcy caused the public trust in possession of Kennedys Bush to allow grazing for income generation. The site was not fenced until 1952, though as late as the 1990's goats were observed scaling the fences (Loughton, 1998), and some ungulate herbivore impact is still observed in the reserve (pers. obs.) The reserve's walking tracks, and proximity to parking and the Sign of the Bellbird (one of Harry Ell's planned rest stops, and one of only four that were actually built) makes Kennedys Bush one of the most well used reserves on the Port Hills.

Omahu Bush

Omahu Bush (also known as Prendergasts Bush) is a privately owned, 60 ha reserve (M 36 787 270) (Wilson, 1994) (Figure A.4). The landowner entered the reserve as a QEII covenant in 1985 and the site is fully fenced (although it still shows evidence of stock intrusion, pers. obs.). With access directly off Summit Road, and well maintained walking tracks and signage, this site is second only to Kennedys Bush for accessibility to the public. Large tracts of the bush consist of regenerating broadleaf or kanuka canopy. However, there is a small pocket of large matai and totara towards the middle of the reserve and some smaller matai close to Summit Road.

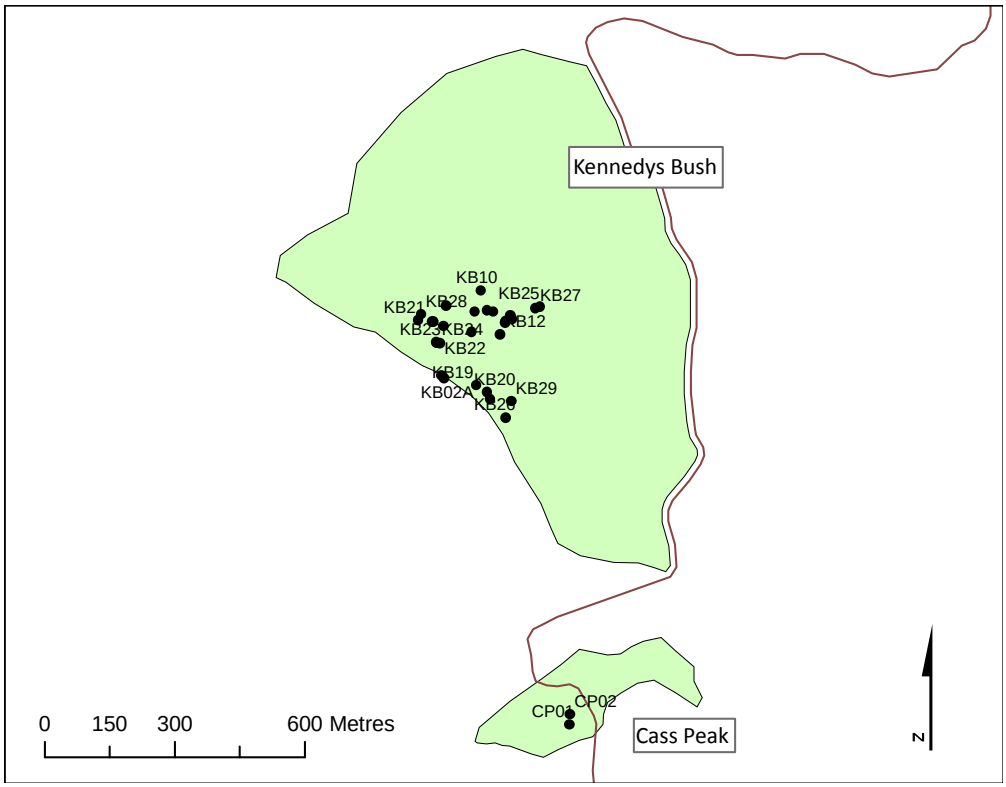

Figure A.3: Mapped adult matai trees in Kennedys Bush and Cass Peak.

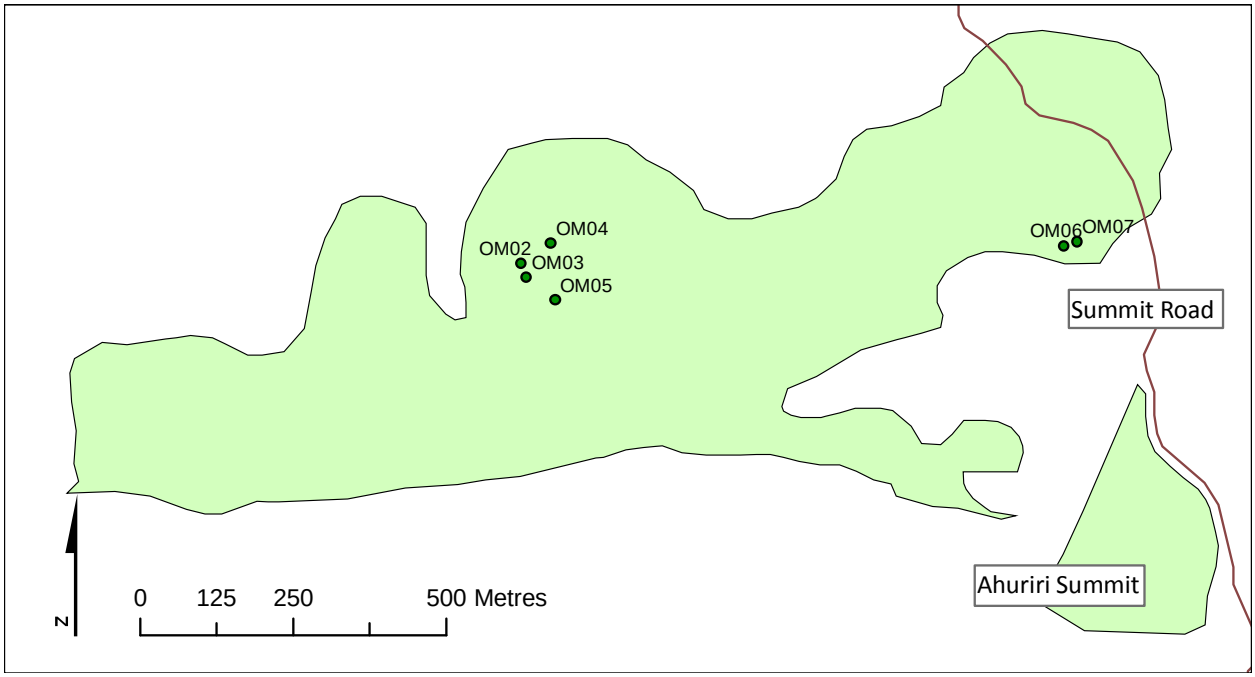

Figure A.4: Mapped adult matai trees in Omahu Reserve.

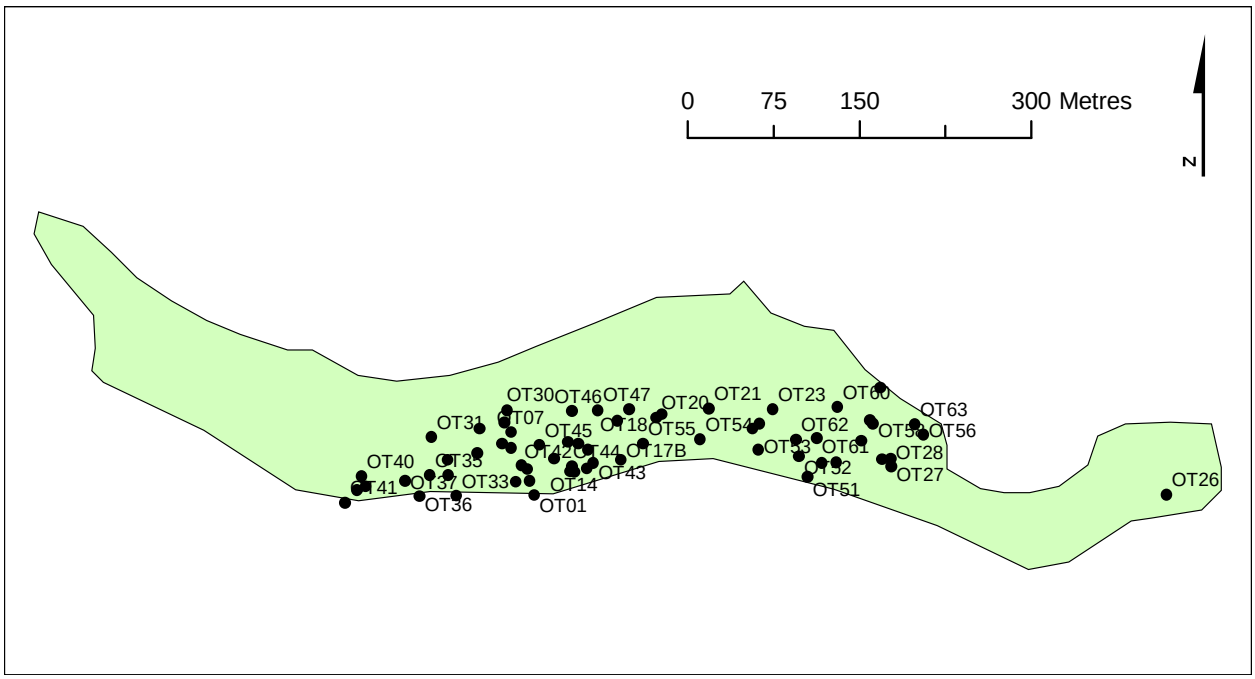

Figure A.5: Mapped adult matai trees in Otahuna Reserve.

Otahuna Reserve

Otahuna Reserve covers an area of c. 40 ha, however only approximately 11 hectares of this consists of mixed broadleaf/podocarp forest (Grid Ref. M 36 783 289) (Wilson, 1994) (Figure A.5). This reserve has only recently been acquired by the CCC, and although the bush is fenced there is a significant effect of herbivory by large ungulates. Adult matai and totara are abundant, but herbivory and the steep nature of the reserve leads to severe erosion, apparently hindering regeneration. Gorse and stinging nettle are common.

Tai Tapu

Tai Tapu is a small (7 ha), privately owned and unprotected forest located on the lower slopes (Table 1.1, Figure 1.1) of the Port Hills (Grid Ref. M 36 764 258) (Wilson, 1994) (Figure A.6). Fencing is incomplete and the bush is heavily used by invasive mammals, including large ungulates (pers. obs.). Despite this, the bush is thick with adult and regenerating matai and lowland totara (Wilson, 1994). Because of the prevalence of high altitude, protected sites across Banks Peninsula and New Zealand as a whole, this site is quite extraordinary for its low altitude and high abundance of podocarps.

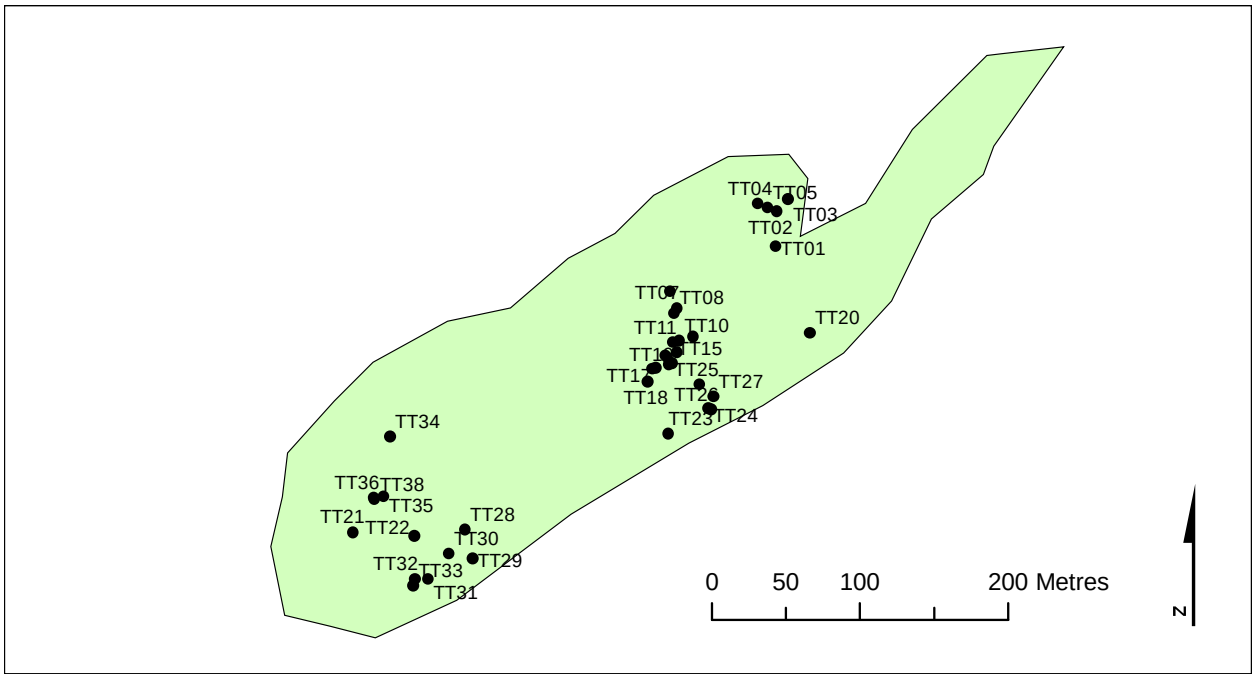

Figure A.6: Mapped adult matai trees in Tai Tapu field site. Note: not all trees have been mapped in this site.

SHORT COMMUNICATION

Seed dispersal of matai (*Prumnopitys taxifolia*) by feral pigs (*Sus scrofa*)

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Abstract: Introduced feral pigs (*Sus scrofa*) include native fruit and seed in their diet, and thus may act as seed dispersers if seeds are passed intact. The aim of this study was to determine whether pigs consume, and subsequently disperse, intact seeds of the New Zealand native tree matai (*Prumnopitys taxifolia*). Two captive pigs were fed 100 ripe fruit of matai and their faeces checked for seeds for 4 days. Fourteen intact seeds (14%) were recovered and 57% of these germinated under glasshouse conditions, comparable with germination from hand-cleaned seeds. We collected 3.5 kg of feral pig faeces from matai-dominated forest in Isolated Hill Reserve, southern Marlborough. This sample contained over 450 intact matai seeds; these seeds readily germinated in the glasshouse, reaching 68% germination after 22 months. These results indicate that pigs are consuming native fruit and passing some viable seeds out – thus acting as occasional seed dispersers.

Keywords: germination; gut passage; Isolated Hill Reserve; mammals; Podocarpaceae

Introduction

Feral pigs (*Sus scrofa*) include fleshy fruits and seeds as part of their diet both in New Zealand and elsewhere (Henry & Conley 1972; Wood & Roark 1980; Thomson & Challies 1988). However, pigs are known to grind up seeds in the process of consumption (Beveridge 1964; Fedriani & Delibes 2009) so this behaviour has rarely been linked to legitimate seed dispersal. Mammals have the potential to be important for long-distance seed dispersal because of their long gut passage times and wide-ranging behaviour (Jordano et al. 2007) and have elsewhere been found to disperse seeds (Abe 2007; Calvino-Cancela 2011; Shiels & Drake 2011). Here we test whether feral pigs are potential seed dispersers of a medium-sized native seed (mataī *Prumnopitys taxifolia*; mean fruit and seed diameters 9 and 7 mm respectively). Mataī is a gymnosperm, so technically produces single-seeded cones, but for simplicity we will use the term 'fruits' for all kinds of fleshy-coated diaspores.

Feral pigs occupy c. 35% of New Zealand, including 13 islands (Fraser et al. 2000). In theory, if pigs disperse a wide variety of native seeds, this could partly compensate for the decline or disappearance of native frugivores (Kelly et al. 2010). Northern Hemisphere diet studies show plant material makes up approximately 80% of the diet of feral pigs. Certain foods are readily consumed seasonally, especially mast seed crops such as acorns and hickory nuts (Henry & Conley 1972; Wood & Roark 1980; Baber & Coblentz 1987; Massei et al. 1996; Schley & Roper 2003).

A New Zealand study of pig diet showed plant material made up 72% of the annual diet, including two fruits (tawa *Beilschmiedia tawa* and hīnau *Elaeocarpus dentatus*) that together comprised 30.9% of the diet (Thomson & Challies 1988). Fruits of mataī, supplejack (*Ripogonum scandens*), miro (*Prumnopitys ferruginea*) and nīkau (*Rhopalostylis sapida*) are also eaten (Beveridge 1964; Knowles & Beveridge 1982; Thomson & Challies 1988). None of these studies recorded whether any seeds were viable, although Beveridge (1964) noted that 'a large quantity' of seeds were crushed.

Although fruit is common in pig diets, studies have been divided on to what extent they destroy the consumed seeds versus passing them out intact. In Hawai'i, pigs disperse viable seeds of the introduced *Passiflora mollissima* (LaRosa 1992). Work in New Zealand confirmed that pigs consume *Passiflora mollissima* fruit and excrete most of the seeds intact and viable (Beavon 2007). In Australia two-thirds of mesquite (*Prosopis pallida*) seeds consumed by feral pigs were undamaged; approximately 80% of these seeds then germinated (Lynes & Campbell 2000). However, in Argentina Campos and Ojeda (1997) found that all *Prosopis flexuosa* seeds found in pig faeces had been destroyed. Consequently, the presence of fruit in pig diets does not necessarily equate to seed dispersal.

We studied the viability of seeds of a native tree, mataī, following consumption by pigs. We hypothesised that pigs consume this native seed and release some intact in faeces, thereby acting at least partially as seed dispersers. Our study has two parts: first we conducted a feeding experiment using captive pigs to determine whether the resulting faeces contained viable seeds; second, faecal samples were collected from a patch of mataī-dominated forest to test whether intact seeds were also present in faeces of wild pigs.

Methods

Captive trial

Two adult, female kunekune pigs (*Sus scrofa*), a New Zealand breed, were kept in captivity at Willowbank Wildlife Reserve, Christchurch, New Zealand. Both were born in captivity and weighed approximately 130 kg. One hundred ripe mataī fruits (one seed per fruit) were fed in one session to the two pigs, with each eating approximately 50 fruits. Most of these fruits were hand-fed to the pigs, with others sandwiched between bread (for easy handling) or placed straight into the feeding trough. All seeds were ingested; none were spat out. Fruits had been collected from the Port Hills, Christchurch, mostly

under a single female mataī, approximately one month earlier, and refrigerated. Only ripe fruits were used.

The entire enclosure was cleaned of pig faeces within 30 min of the seeds being ingested. Pig faeces were then collected during daily cleaning for four consecutive days. These samples were taken back to the laboratory and sifted to recover seeds.

Field study

Field evidence of pig dispersal was collected in winter 2008 from Isolated Hill, a 2835-ha scenic reserve in southern Marlborough, New Zealand. Mataī-dominated forest makes up 8% of the reserve (Williams 1982). Feral pigs are present throughout the reserve, being especially abundant in areas containing mataī (Cochrane 1994). We observed on initial visits that pig faeces in this area appeared to contain large amounts of crushed mataī seeds. On a single day in June 2008 a 3.5-kg sample of pig faeces was collected from under c. 12 female mataī trees through a 1-ha area of mataī-dominated forest within the reserve (E2591170, N5924350, NZMG). Although pig faeces were most easily collected under female mataī trees, given the long gut passage times of pigs (see Results), mataī seeds are likely to be present in faeces deposited elsewhere. The samples were collected 1–2 months after mataī had finished fruiting; all faeces were solid and water was required to sift samples and retrieve seeds.

Germination

All intact seeds recovered from both the captive and field trials were potted in June 2008 and kept under glasshouse conditions as for previous studies (Kelly et al. 2010) at the University of Canterbury, Christchurch, New Zealand, for two spring/summer cycles until June 2010. Germination was recorded at least monthly, but weekly during the main period of germination (November to March each year for 2 years). We were unable to measure the germination rate of non-pig-processed seeds ourselves, as all the available fresh fruits collected were used in the captive feeding trials. We therefore compared our data to hand-cleaned and germinated mataī seeds presented by another study (Kelly et al. 2010, their table 2). The same glasshouse was used as for Kelly et al.'s study, but their seeds were sourced from elsewhere in the northern South Island, at Pelorus Bridge (Nelson) and Blue Duck Reserve (Kaikoura). In addition, we potted 20 seeds from Isolated Hill that were partially damaged from pig mastication. Overall germination success rates were compared between trials using chi-square tests on the totals.

Results

Captive trial

The captive pigs readily ate the mataī fruits offered to them, consuming all within 5 min. The pigs made loud crunching sounds as they chewed the seeds. Despite this, of the 100 seeds fed to the pigs, 14 intact seeds were retrieved from the collected faeces. The seeds were excreted over 4 days, with most collected on Day 3. In total 8 of the 14 seeds (57%) germinated in the glasshouse – seven in spring 2008, one in spring 2009 (Table 1). As the Day 4 sample still contained some mataī seed we cannot exclude that some seeds were excreted after the trial ended. The faeces contained an unquantifiable amount of shattered mataī seed coat; no partially damaged seeds were recovered.

Field study

The 3.5 kg of pig faeces collected from Isolated Hill contained abundant mataī seed-coat fragments (not quantified), many intact mataī seeds (c. 470), and some damaged seeds: 20 partially damaged mataī seeds (potted in glasshouse) and 20 mataī seeds broken in half with traces of the embryo present (not potted). If we apply the 14% recovery rate of intact seeds from the captive trials, then this would suggest approximately 3400 fruits were consumed by the pigs in question. No other food sources were identified in the faecal samples, indicating pigs were concentrating on mataī fruit in the area sampled at that time. There were no intact seeds from other plant species.

Germination

Glasshouse seeds began germinating in November 2008, 6 months after being planted. Germination continued throughout the next year, with low but consistent numbers over winter, followed by a second flush beginning in September 2009. No further germination was recorded after March 2010 (22 months after the seeds were planted). All three samples (hand-cleaned, captive, field) reached approximately 60% germination. We compared total germination success between the hand-cleaned seeds (from Kelly et al. 2010), captive trial and field trial. There was no significant difference between hand-cleaned seeds and either captive trial data ($\chi^2 = 0.04$, d.f. = 1, $P = 0.85$) or field data ($\chi^2 = 1.33$, d.f. = 1, $P = 0.25$). An additional 8 seedlings germinated from partially damaged seeds obtained from feral pig faeces.

Table 1. Germination tests on seeds of mataī (*Prumnopitys taxifolia*) that have passed through captive and feral pigs. Seeds were sown in a glasshouse (University of Canterbury, Christchurch, New Zealand) and germination recorded over two spring–summer cycles.

	Captive pig trial	Feral pig study		Hand-cleaned (Kelly et al. 2010)
	Intact seeds	Intact seeds	Partially damaged	
No. planted	14	472	20	200
No. germinated				
Spring–summer 1	5	115	3	114
Spring–summer 2	3	208	5	13
Total germinated (*n*)	8	323	8	127
Total germinated (%)	**57.1**	**68.4**	**40.0**	**63.5**

Discussion

This study shows that feral pigs consume mataī fruits in the wild and deposit a proportion of intact, viable seeds following gut passage. Though pigs are known to disperse introduced weeds (Beavon 2007), this appears to be the first New Zealand report of viable native seeds being dispersed by pigs.

Generally, pigs have been thought to be largely nomadic (Wodzicki 1950); however, studies both in New Zealand and elsewhere have shown distinct home ranges and small daily travel distances (Martin 1975; McIlroy 1989; Saunders & Kay 1991; Caley 1997). New Zealand studies showed home ranges up to 2 km^2 and immature pigs moving up to 3 km in a 24-hour period (Martin 1975; McIlroy 1989). Mammalian home ranges can be much larger than those of birds (Jordano et al. 2007).

This slow gut passage of seeds (2–4 days) has large implications for potential dispersal. With pigs possibly covering several kilometres in a 24-hour period (McIlroy 1989), seeds could be moved kilometres away from the parent tree. Also significant is that seeds were defecated over the space of 3 days. Rather than all seeds consumed at the same time being defecated in one spot, they could be spread over a larger area, reducing spatial clumping of seeds. Long gut passage times in pigs may negatively affect seed germination, as has been found in other studies (Fedriani & Delibes 2009), but no such lowered germination was found in mataī when compared with hand-cleaned seeds. This may be due to the thick endocarp of mataī (Thorsen et al. 2009).

It is possible that other introduced mammals may provide some dispersal services to native plants, as demonstrated for ship rats (*Rattus rattus*) in Hawai'i (Shiels & Drake 2011) and Japan (Abe 2007), rabbits (*Oryctolagus cuniculus*) in Australia (Calvino-Cancela 2011), and carnivores (badgers, foxes and stone martens) in Spain (Jordano et al. 2007). In New Zealand, ship rats passed only very small seeds intact (Williams et al. 2000), while brushtail possums (*Trichosurus vulpecula*) have been found to include small fruits in their diet, and excrete intact and viable seeds (Dungan et al. 2002). Mataī seeds have been retrieved from possum faeces and germination percentages of 40–50% recorded (J. Ladley, University of Canterbury, pers. comm.). However, the importance of possums as dispersers of larger native fruit is still controversial (Williams 2003). There is little information about possible seed dispersal by larger introduced mammals such as deer or feral cattle (Kelly et al. 2010).

While pigs may make a relatively small numerical contribution to dispersal of native seeds, in New Zealand's modified environment this may assist in maintaining dispersal. The loss of native avifauna has been well documented (Bell 1991), and the implications for mutualisms discussed and studied (Clout & Hay 1989; Kelly et al. 2004, 2010). In several cases it appears that, despite reduced densities of native birds and little contribution by introduced birds to fruit dispersal (Kelly et al. 2006), adequate dispersal service is still being provided to native plants (Kelly et al. 2010; Wotton & Kelly 2011). However, pigs could provide an important addition to the current suite of dispersers, given their likely long-distance dispersal. This is similar to the study of Jordano et al. (2007), which showed that c. 85% of dispersed *Prunus mahaleb* seeds in Spain were moved by frugivorous birds, but that terrestrial mammals (badgers, foxes and stone martens) contributed 67% of long-distance dispersal events.

This study has shown that feral pigs are consuming and passing intact viable seeds of one New Zealand species. Coupled with daily movement across several square kilometres, feral pigs are probably important in the long-distance dispersal of seeds, both of native plants and of introduced weeds.

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